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A Collective Memory Event

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With a foreword by **Grahame Bulfield**

THE UNIVERSITY *of* EDINBURGH

BBSRC
bioscience for the future

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Old College, University of Edinburgh • 19 April 2016

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Foreword

GRAHAME BULFIELD
Director of Roslin Institute, 1988–2002

The publication of the research paper in 1997 on the cloning of the first animal from an adult cell – Dolly the sheep – caused a scientific and media storm. The scientific breakthrough, long thought to be impossible, was achieved by a small team from Roslin Institute led by Ian Wilmut together with colleagues from its spin off company PPL Therapeutics.

This is a transcript of a Collective Memory Event held on the 19th April 2016 with members of the team and other colleagues closely involved. Of course, although 1997 is seen as the date of the publication of the research, its origins lie much earlier, not only with the birth of Dolly in 1996, the cloning of Megan and Morag from embryo cells in 1995, but also with the long standing interest of Ian Wilmut and his group in the potential totipotency of somatic cells and in the derivation of stem cells. Indeed, I first heard in detail of Ian's research objectives in December 1988, a few days after I became Head of Roslin,[i] nearly a decade before the publication.

The question I was most asked at the time was 'why is Roslin doing this research?' It appeared strange, if not incomprehensible, for a small agriculturally-oriented Institute in Scotland to be working on such a challenging topic, which seemed, at first glance, to have no practical relevance. In the late 1980s and early 1990s, the molecular biology revolution was sweeping through biological research. The first genetically modified/transgenic animal (a mouse) had been produced and the beginnings of the genomics revolution were in place. Roslin, like many other biological research institutes, decided it had to adopt these technologies, especially as no one else was doing so in farm animals, which is where Roslin's expertise lay. Transgenic sheep had been produced in Roslin and a company, PPL Therapeutics,[ii] was established to exploit the technology, although primarily in producing pharmaceuticals rather than for animal breeding. Fortunately as a government sponsored Institute, Roslin (in collaboration with John Innes Institute) managed to persuade their parent organisation and main funder, the BBSRC,[iii] to establish decade-long research programmes in transgenics and stem cells, and in gene mapping and genomics.

For routine use in animal breeding, there were problems with transgenic technology, especially that it was too inefficient. Less than one per cent of injected eggs produced viable transgenic offspring and almost all commercially important traits in farm animals are controlled by many genes, whose number, identity and characteristics are all unknown.[iv] It was to improve transgenic technology that Ian Wilmut proposed using nuclear transfer from somatic cells; the programme was funded under the new BBSRC initiative and Keith Campbell was appointed as the post-doc on the project. The first cells targeted were from a stable embryo cell line isolated by Jim McWhir, and produced Megan and Morag, the first cloned sheep, in 1995. Next year, the group's main aims were to repeat the experiment with embryo cells but also foetal fibroblasts, which they thought to be the most suitable cells for manipulation. It was only because extra sheep were available and a suitable mammary cell line had been produced at PPL, that the team had adult cells that could be used to produce Dolly.

This Collective Memory Event brings together most of the scientists involved in the experiments: Ian Wilmut who led the team, Bill Ritchie who carried out the embryo manipulations and John Bracken who cared for the animals, together with scientists from PPL Therapeutics, Alan Colman, its Research Director, and

Angelica Schnieke who produced the mammary cell line. They are joined by other Roslin colleagues: Harry Griffin who was at the time Assistant Director of Science (and later Director); Alan Archibald, leading Roslin's genomics programme; Bruce Whitelaw leading mammalian transgenics; and Helen Sang leading poultry transgenics. The event was chaired by Robin Lovell-Badge, a leading figure in transgenesis who was also a member of the Roslin Governing Council at the time when these events took place. These are then the genuine voices of what happened at Roslin in the mid-1990s and their recollections make a fascinating story of not only the scientific research but also the complex interactions that took place. It is only so very sad that John Clark and Keith Campbell, so much leading players in these events, were no longer with us and able to take part.

The cloning of Dolly was in some ways a diversion from what Roslin was trying to achieve; later experiments did in fact produce cloned animals from manipulated foetal fibroblasts cells with working human genes and capable of producing pharmaceuticals, but this success got lost in the furore of Dolly's arrival. Although the farm animal genomics programmes have now identified many genes controlling commercially important traits, the political climate has strongly inhibited the use of GM technology in practical animal breeding.

The Dolly publication also generated much public, media and government interest. Roslin was overwhelmed with TV crews and request for interviews; myself and Ian Wilmut were called to a special meeting of the House of Commons Select Committee on Science and Technology that was squeezed in a few weeks later, just before the 1997 General Election. The ethics of the technology were debated at the Human Genetics Advisory Commission in the UK and at by various bodies in the EU, USA and the rest of the world. One positive side of all this attention was that Roslin's policy of openness about its research (mainly executed by Harry Griffin) turned out to be very successful and Roslin became seen as a 'safe pair of hands'; we were asked for our opinion by the media and Government on many biotechnology issues, not all in our area of expertise. This interest continues up to the present.

In 1998 Roslin established a second company, Roslin BioMed, with £6M of funding from the 3i Venture Capital Group, in order to develop the cloning technology. It was the multimillion pound sale of Roslin Biomed to the Californian biotech company, Geron, that stimulated more political interest. Roslin affairs were examined by the National Audit Office; myself and Harry Griffin had to attend the House of Commons Public Accounts Committee to explain and justify our actions. The money Roslin received from the sale to Geron was put into a Foundation, which continues to fund research at Roslin.

The story of 'Dolly the Sheep' is one of a formidable piece of research with a seemingly impossible objective, outstanding success from the persistence and skill of an excellent team of scientists, to its explosion into the public domain which engulfed those involved, producing many lessons, not only of science strategy, but also of the role of scientists in their interaction with commercial companies, the Government and the public; the voices of those involved add much to the story.

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i. I have used the name Roslin Institute throughout this foreword, but although its origins go back to 1919, the Institute only adopted its current name in 1993.

ii. PPL Therapeutics Ltd. was founded in 1987 as Caledonian Transgenics, but was soon renamed Pharmaceutical Proteins, Limited, and eventually, PPL.

iii. The Biotechnology and Biological Sciences Research Council (BBSRC), the 'owners' of Roslin Institute at this time, was only founded in 1994, although its origins go back to the formation of the Agricultural Research Council in the 1930s.

iv. A solution to the gene identity problem came from the genomics programmes.

Introduction

DMITRIY MYELNIKOV & MIGUEL GARCÍA-SANCHO
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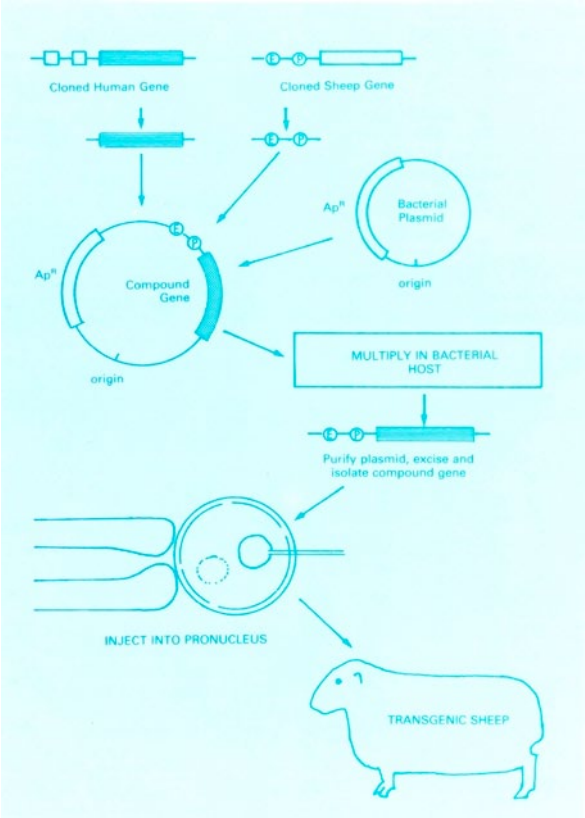
Born on 5 July 1996, Dolly the sheep was the first animal to be cloned from an adult cell, and became the most iconic member of her species. Many accounts of her creation already exist, authored by some of the scientists involved as well as journalists and social scientists.[i] As part of the BBSRC-funded 'Historicising Dolly' project at the University of Edinburgh, we sought to conduct a group memory event that would gather different members of Roslin Institute and PPL Therapeutics who were either involved in the cloning or observed it as colleagues. Our purpose was twofold. First, we sought to place Dolly within the broader genetic engineering research programme at Roslin, alongside other cloned and transgenic sheep made there, and in the historical context of 1990s biotechnology and science funding. Second, through a collective memory event, we hoped to gather diverse perspectives and allow the participants to reflect on the established narratives together. What follows is a brief introduction to the Roslin Institute and some of the key experiments discussed here, which should help the reader follow the transcript.

From ABRO to Roslin

Roslin Institute traces its history to the Animal Breeding Research Department at the University of Edinburgh, set up in 1919 with F. A. E. Crew as its first director. It soon became the Institute of Animal Genetics and was a major site in the budding discipline of genetics, with focus on farm as well as laboratory animal heredity. After World War II, the UK Agricultural Research Council (ARC) founded the Animal Breeding Research Organisation (ABRO) on the basis of the Institute. ABRO's mission was to pursue basic and applied research into farm animals to improve breeding programmes in the nation. The organisation was showing a steady growth into the 1970s, when the reforms following the split its funding between the block grant from the ARC and research contracted by the Ministry of Agriculture, Fisheries and Food. The split stemmed from Lord Rothschild's controversial 1971 report that became government policy under Edward Heath, instilling a 'customer-contractor' principle on applied R&D.

In 1982, ABRO faced a serious crisis. The ARC, eager to pursue biotechnology research and make savings, threatened an 80% cut to the Organisation. The proposal was met with resistance, and farm groups, media outlets and a few MPs rallied to support the Organisation; eventually, ABRO lost about 50% of its funds, but promised to reorient its research towards genetic modification. Serious cuts to the ARC under Margaret Thatcher's government followed shortly after, and many council institutes were merged, shut down or privatised. While ABRO was spared the more dramatic fate, in 1986 it was merged with the Roslin-based Poultry Research Centre, and the Babraham Institute of Animal Physiology near Cambridge. The new mammoth Institute of Animal Physiology and Genetic Research (IAPGR), with an Edinburgh and Cambridge Research Stations, lived for eight years, and was split into Babraham and Roslin Institutes in 1993. By then, the Edinburgh scientists had consolidated their research at a former Poultry Research Site in the village of Roslin. In 2008, the Institute was incorporated into the University of Edinburgh, and in 2011 moved into a brand new building at the Easter Bush Campus just outside Roslin



THE PHARMING PROGRAMME

The diagram in Figure 1 shows how to make pharmaceutical proteins in sheep milk ('pharming'). A human gene of interest — for example, the gene for alpha-1-antitrypsin, an emphysema drug that PPL pursued — that had been isolated and spliced with sheep enhancer (E) and promoter (P). These are the DNA elements that regulate gene expression, i.e. how much protein is made from DNA, and in which tissue – here, the mammary tissue is targeted to make the protein in milk. The human/sheep construct is inserted into a circular bacterial DNA molecule, or plasmid, which is taken up by bacterial cells, and multiplies in them as the bacterial colony grows. The modified plasmid can then be purified, and the compound human-sheep gene isolated. This resulting DNA molecule can then be microinjected into the shep embryo at a 1-cell stage, i.e. a fertilised sheep egg. The injection happens into one of the pronuclei – a sperm or egg nucleus that do not merge until the embryo divides for the firtst time. If things go well, the resulting sheep will be transgenic, or carry the inserted gene and make the desired protein in its milk.

FIGURE 1. Diagram showing the making of transgenic sheep, from the ABRO 1985 annual report, p. 22. Courtesy of the Main Library, University of Edinburgh.

From pharming to cloning

As part of the 1982 settlement with the ARC, ABRO embraced genetic modification and hired scientists with expertise in molecular biology. By that point, genetically modified or transgenic mice, first reported in 1980, were being adopted by labs across the globe, and there were hopes that similar techniques could be applied to farm animals. Rick Lathe, who had worked at a biotechnology company Transgéne in Strasbourg, was hired in 1984. Together with John Bishop at the Department of Genetics, and John Clark, Bishop's post-doc, Lathe devised the programme of making transgenic sheep. Rather than focus on improving breeds with genes – a prospect that was proving difficult for key US groups – Lathe and Bishop pursued production of pharmaceutical proteins in milk instead. The idea echoed the business model of new US biotechnology firms like Genentech, which pursued protein production in bacteria. For Lathe, farm animals offered two advantages: some human proteins could not be processed properly in bacterial cells, and milk production promised high yields. This idea came to be known as 'pharming' (portmanteau of 'pharmaceuticals' and 'farming').

Lathe left ABRO shortly after, in 1986, and John Clark took over the programme. By that point, transgenesis in sheep had not been achieved anywhere, and serious embryological expertise was required (see *Fig. 1*). Ian Wilmut, who had joined ABRO in 1973, was diverted from his research on prenatal mortality to help with embryo injections and transfer, and Paul Simons was hired to perform the hands-on manipulations. Alan Archibald, another ABRO scientists who had recently trained in molecular skills at the European Molecular Biology Laboratory in Heidelberg, made many of the gene constructs.

Meanwhile, faced with funding gaps, ABRO management sought to seek private investment, in line with Thatcherite science policy. With the help of the Scottish Development Agency, a local body set up with North Sea oil money to boost business in Scotland, and private investment funds, a new company was

founded in 1987 to commercialise pharming. First called Caledonian Transgenics, it soon changed its name to Pharmaceutical Proteins Limited, or PPL. PPL funded considerable amounts of the molecular research at ABRO's successors, IAPGR and Roslin Institute, in exchange for licensing the patents, and set up its own research programmes in parallel.

In 1990, the pharming programme celebrated its first big success, as Tracy the sheep was born at Roslin. While not the first transgenic sheep to be made, Tracy produced vast amounts of alpha-1-antitripsin, a protein used in treatment of emphysema and cystic fibrosis, in her milk. Despite this success, production of transgenic sheep remained difficult, slow and expensive, many manipulated embryos did not deliver, and where the foreign DNA would integrate within the sheep genome was left to chance. Again, mouse researchers paved the way. In 1989, a first 'knockout' mouse was born, which had a gene removed rather than added. The result was made possible by using embryonic stem cells – first cultured in 1981 – that could be manipulated in culture, with a lot more ease and reliability. Sheep embryonic stem cells were not available, but in the early 1990s, Roslin and PPL invested considerable resources in working with cultured cells.

The project to clone sheep from somatic, i.e. differentiated body cells developed from this interest, and envisioned cloning as a way to reproduce successfully modified sheep without access to stem cells. While Dolly was not genetically modified, a sheep called Polly was born in 1997, both cloned and transgenic. In a sense, Dolly was a prototype, and Polly the pinnacle of the project, but this second birth was eclipsed by the furore that was Dolly. Yet, as this transcript makes clear, there were other interests at play in the making of Dolly. Thus, the Ministry of Agriculture, Fisheries and Food (MAFF) sponsored a significant part of the research, but was eager to keep at an arms length from controversial and commercially-oriented genetic engineering. Instead, applications to the Ministry emphasised potential of reproductive cloning of valuable farm animals. Finally, the scientists at Roslin were also interested in what cloning could reveal about embryonic development and differentiation.

Cloning

Cloning has been a charged and fascinating topic for biology and its various publics. In 1952, Robert Briggs and Thomas King, based at the Institute for Cancer Research of the Lankenau Hospital Research Institute near Philadelphia, cloned a frog *Rana pipiens* from undifferentiated embryonic cells. They relied on nuclear transfer – transplantation of a cell nucleus into a recipient egg cell whose nucleus had been removed. In 1959, using a different frog species, *Xenopus laevis*, John Gurdon at Oxford University managed to clone frogs from differentiated tadpole cells. These experiments answered important questions about how genes persisted in development, suggesting cells did not lose genetic material as they divided, but rather that differentiation was achieved by switching genes on and off.

Cloning in mammals, however, proved difficult. In 1981, Karl Illmensee at the University of Geneva reported cloning three mice from embryonic cells, but after allegations of fraud from within his team this claim was dismissed. New discoveries about parental imprinting – the idea that both paternal and maternal chromosomes contributed crucial epigenetic information, and thus both were necessary for successful development – were being made in the early 1980s, and cloning from adult cells seemed unlikely. Yet while there were many setbacks in mice, nuclear transfer was increasingly promising in agriculture. At the Cambridge Animal Research Station,[ii] Steen Willadsen cloned a sheep from early embryonic cells and published the result in 1985. At the University of Wisconsin, Neal First's group paved the way with cloning cattle from embryonic cells.

Roslin succeeded in cloning sheep from cultured embryonic cells in 1995, when Megan and Morag were born, to a significant, but mostly forgotten, controversy. They were made from the TNT cell line that offered a promise of genetic manipulation. Following these experiments, Roslin and PPL scientists pursued other kinds of sheep cells – foetal fibroblasts and mammary cells – to confirm whether a cell's embryonic status was essential for the procedure. In 1996, Dolly was made from a nucleus that came from the mammary cells of a Finn Dorset sheep – long dead by then – that was transplanted into an enucleated oocyte of a Scottish Blackface sheep. The nucleus and oocyte were fused with an electric pulse, and the resulting embryo was implanted into another Scottish Blackface surrogate mother.

On 5 July 1996, Dolly was born. While the scientists were ascertaining her genetic origins and writing up the paper, she was kept secret, even from most staff at Roslin, as this transcript shows.

FIGURE 2. Dolly shortly after her birth, a Finn Dorset lamb next to her Scottish Blackface surrogate mother.
© Roslin Institute.

Legacy

Dolly was due to be announced on 27 February 1997, to coincide with the publication of the scientific paper in *Nature*. *The Observer* broke the news a few days early, on 22 February, derailing Roslin and PPL's careful publicity plans that had been organised by the PR firm De Facto. The havoc that ensued was driven by immediate associations with human cloning, and it was fuelled by government interest in both the UK and the United States – Bill Clinton's newly-appointed head of the National Bioethics Advisory Commission was among the first people to phone Roslin. Multiple ethical reports condemning human cloning were published, and the UK parliament rushed to adjust the Human Embryology and Fertilisation Act to explicitly ban human cloning.

At first, the controversy seemed dangerous for Roslin. Ethical concerns made scientists worry that cloning experiments could be banned altogether. MAFF, already reluctant to deal with genetic modification and weary of the controversy, had rushed its plan to shut down funding for cloning at Roslin. Yet despite this initial uncertainty, the Institute used Dolly to its maximum advantage. Grahame Bulfield appealed to the scientific press and MPs about the defunding of what emerged as a key site for global biology, and MAFF reversed its decision. The patents derived from cloning – the cell culture methods rather than the nuclear transfer per se – were not covered by the agreement with PPL, and Roslin decided to exploit them more aggressively. A new company, Roslin Biomed, was established in 1998 and sold to the US biotech firm Geron the next year. The deal funnelled much-needed investment into the Institute, ending years of austerity. Finally, Dolly herself proved an asset. Friendly and happy to engage with humans – as she learned to expect a treat in return – Dolly came to represent a softer edge of biotechnology.

This transcript adds to the existing narratives around Roslin in several ways. First, it highlights the roots of the cloning programme within pharming and shows that they stemmed from the 1980s policies about agricultural science. It hints at alternative research programmes – making pharmaceuticals in eggs, for example – and highlights collaboration both within Roslin and between the Institute and PPL. Finally, it offers multiple perspectives on the legacy of Dolly's

birth for Roslin, but also for agricultural sciences, biomedical research, the biotech industry and the science-media relations.

Note on the transcript

The event transcribed here took place on 19 April 2016, in the picturesque Elder Room, Old College, University of Edinburgh. The method we pursued was that of a witness seminar, which aims to bring together multiple perspectives as participants share, question and clarify their memories, with minimal interference. The Wellcome Trust Witnesses to Twentieth-Century Medicine series, organised and edited by Tilli Tansey and her team, was the major inspiration.[iii] This event was different in that it focused on a single institution rather than a field. We decided to limit the audience to the scientists and technicians from Roslin and not invite external participants such as journalists involved in announcing Dolly, to create a relaxed atmosphere where the witnesses would not be immediately encouraged to adopt a public-facing persona.

The event went on for about four hours, split in two sessions. The conversations was recorded and transcribed, and the transcript sent to participants for edits and clarifications. We have then incorporated their (very minor) changes into this version, alongside light editing for style and clarity, trying to maintain the conversational nature of the event while making it easy to follow as written prose. The opinions of the speakers are their own.

The text is annotated in two ways. Endnotes are used to cite the literature, clarify certain passages and offer context. In parallel, definitions of the more obscure scientific terms are given in the margins the first time they appear in text. Despite the occasional esoteric passage, we hope that this transcript can do some justice to the palpable excitement that was felt on the day of the event.

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i. An account of Dolly's cloning co-authored by Ian Wilmut and Keith Campbell, the team leaders, was published as Ian Wilmut, Keith Campbell & Colin Tudge, *The Second Creation: Dolly and the Age of Biological Control* (Cambridge, MA: Harvard University Press, 2001). For a journalist investigation published very shortly after Dolly became public knowledge, see Gina Kolata, *Clone: The Road to Dolly and the Path Ahead* (London: Penguin, 1998). For an anthropologist's perspective that places Dolly in multiple contexts, from biocapital to imperialism, see Sarah Franklin, *Dolly Mixtures: The Remaking of Genealogy* (Durham, NC: Duke University Press, 2007). Various sources and essays on cloning are collated in Arlene Judith Klotzko (ed.), *The Cloning Sourcebook* (Oxford: Oxford University Press, 2001). For a historical account that situates Dolly within the genetic engineering programme at Roslin, see Miguel García-Sancho, 'Animal Breeding in the Age of Biotechnology: The Investigative Pathway behind the Cloning of Dolly the Sheep,' *History and Philosophy of the Life Sciences*, 37 no. 3 (2015): 282–304. On the history of Roslin's precursor institutions and the 1980s cuts, see Dmitriy Myelnikov, 'Cuts and the Cutting Edge: British Science Funding and the Making of Animal Biotechnology in 1980s Edinburgh,' *British Journal for the History of Science* (forthcoming); see also the Towards Dolly archive blog http://libraryblogs.is.ed.ac.uk/towardsdolly, last accessed on 3 April 2017.

ii. The Animal Research Station in Cambridge was an ARC unit where major work on developmental biology in farm animals, as well as mice, took place. In 1986, in the merger with ABRO to make IAPGR, the Station was merged with the Babraham Institute of Animal Physiology and thus staff who remained became part of IAPGR. See Chris Polge, 'The work of the Animal Research Station, Cambridge,' *Studies in History and Philosophy of Biological and Biomedical Sciences* 38, no. 2 (2007): 511–520.

iii. On the witness seminar methodology, see E. M. Tansey, 'Witnessing the Witnesses: Pitfalls and Potentials of the Witness Seminar in Twentieth Century Medicine,' in *Writing Recent Science: The Historiography of Contemporary Science, Technology and Medicine,* ed. Ron Doel & Thomas Soderqvist (London: Routledge, 2006), pp. 260–78; E. M. Tansey, 'The Witness Seminar Technique in Modern Medical History,' in *Social Determinants of Disease*, ed. Harold J. Cook, Sanjoy Bhattacharya & Anne Hardy (Telangana: Orient Longman, 2009), pp. 279–295. See catalogue of available witness seminars at www.histmodbiomed.org/article/wellcome-witnesses-volumes/, last accessed on 3 April 2017.

Timeline of key events

1947	Animal Breeding and Genetics Research Organisation is founded by the Agricultural Research Council (ARC) on the basis of the Institue of Animal Genetics. Renamed Animal Breeding Research Organisation (ABRO) in 1951.
1981–82	The ARC proposes an 80% cut at ABRO, driven by budgetary pressures and wish to free more funds for high-priority research, including biotechnology. After much resistance, about 50% of ABRO is cut, but extra funding is made available for genetic engineering research.
1983	The ARC becomes the Agricultural and Food Research Council (AFRC).
1986	With further cuts to AFRC, ABRO is merged with the Poultry Research Centre in Roslin, and the Babraham Institute of Animal Physiology in Cambridgeshire, to form the Institue of Animal Physiology and Genetics Research (IAPGR).
1987	Caledonian Transgenics Ltd. is set up to commercialise pharming research in Edinburgh. It soon becomes Pharmaceutical Proteins Limited (PPL).
1990	Tracy the transgenic sheep is born at IAPGR.
1993	Roslin Institute is established after IAPGR is split.
1994	AFRC is merged with parts of the Science and Engineering Research Council to become the Biotechnology and Biological Sciences Research Council (BBSRC).
1995	Megan and Morag are cloned from cultured emrbyonic cells.
5 July 1996	**Dolly the Sheep is born.** Her cloning is kept secret until publication.
22 February 1997	Dolly is announced by *The Observer*, days before the scientific article is due to appear in *Nature*. Media frenzy ensues.
1997	Polly and Molly, who are both cloned and transgenic, are born at Roslin.
1998	Roslin BioMed is founded to commercialise cloning and related techniques.
1999	Roslin BioMed is sold to Geron, a US biotechnology company.
2003	Dolly is put down after she develops jaagsiekte (viral lung cancer).
2008	Roslin Institute becomes part of the University of Edinburgh.

Participants

Alan Archibald was one of the ABRO scientists to adopt molecular tools in the 1980s, and was closely involved in the pharming programme and the Pig Genome Project. He is currently the Deputy Director at Roslin.

John Bracken was an animal carer and anaesthetist at Dryden farm, Roslin Institute, and looked after Dolly since her birth. He is currently retired.

Alan Colman was the Research Director at PPL Therapeutics. He is currently a Visiting Fellow at Harvard University.

Harry Griffin was the Assistant Director for Science at Roslin, and one of the spokespeople for the intsitute when Dolly became public. He is currently retired.

Robin Lovell-Badge chaired the event. He has worked with mouse embryonic stem cells and transgenesis, and was on the Roslin Governing Council. He is currently a greoup leader at the Crick Institute in London.

Angelika Schnieke was a scientist at PPL Therapeutics and was involved in devising the cell line used for Dolly's cloning. She is currently Professor of Livestock Biotechnology at the Techniche Universität in Munich.

Jim McWhir was a cell biologist at Roslin, who was involved in culturing the cells for Dolly and other sheep. He is now retired.

Bruce Whitelaw joined IAPGR in 1987 and was involved in the pharming programme. He currently heads the Division of Developmental Biology at Roslin.

Bill Ritchie is an embryologist who performed the micromanipulations for Dolly's cloning. He is the founder of Roslin Embryology, Ltd.

Sir Ian Wilmut was the leader of the Roslin team that cloned Dolly. He is currently Professor Emeritus and Chair at the Scottish Centre for Regenerative Medicine, University of Edinburgh.

Helen Sang joined the Poultry Research Centre in 1984. She is an expert in genetic modification of poultry and Professor of Vertebrate Molecular Development at Roslin.

FIGURE 3. Participants on the day of the event. *Left to right*, Bruce Whitelaw, Alan Archibald, Bill Ritchie, Harry Griffin, Helen Sang, John Bracken, Miguel Garcia-Sancho, Jim McWhir, Angelika Schnieke, Alan Colman, Ian Wilmut and Robin Lovell-Badge. © Dmitriy Myenikov

The transcript

1. Roslin, PPL, and the pharming programme

Robin Lovell-Badge. We need to try and get some ideas of the context that Dolly was created in. Obviously there are different aspects to this context and so there's the various research that led up to the possibility of cloning animals. Beginning with frogs, and then there's various bits of science done in various animals that gradually sort of progressed the field. In mice there were the rather dubious experiments by Karl Illmensee, there were new methods developed by James McGrath and Davor Solter, which were obviously very instrumental.[1] Of course there was the discovery of *parental imprinting*, which some people, in particular Solter, thought that would make it impossible to clone animals, or clone mammals. Then there were people working in cows, like Neal First, and then there were people like Steen Willadsen, who did some early experiments trying to clone sheep, using nuclei from the early embryos.[2] So that was actually in 1986, taking nuclei from 8- or 16-cell embryos and putting them into unfertilised *oocytes*, which was interesting, because most of the work that was going on, in mice at least, was trying to use fertilised eggs. So there are questions like why were sheep cloned first? The importance of the expertise at Roslin, collaborations, which we hope we will get from you.

Parental imprinting refers to certain genes being expressed in a parent-of-origin-specific manner. In the 1980s, consensus emerged that normal development requires the contribution of both the maternal and paternal genomes.

An *oocyte* is a precursor of the egg cell (ovum).

I should also actually say at the outset that of course there are a few key people who aren't here. Obviously some can't be, and the two obvious ones are John Clark and Keith Campbell, so I think you said you would appreciate if anyone has views that they think those two individuals would have, to express them.[3] So, the first question that I was asked to put forward is 'It's sometimes said that the cloning of Dolly was an accident or a by-product, in the sense it took place within the pharming programme, and that programme sought the production of *transgenic* sheep. So why produce cloned sheep within a research programme that sought the genetic modification of animals?' I don't know who wants to tackle that question. Ian?

A *transgenic* organism carries foreign DNA introduced experimentally that has integrated into its genome.

Ian Wilmut. Yes, I'll have a go. There was actually a student who'd completed a PhD at Roslin, Lawrence Smith, he was from Canada. I guess he did similar things to First, he produced a very small number of lambs after nuclei transfer from early embryos...

The initiative for the larger-scale, cloning project came, I think, when I heard at a meeting from a colleague of Willadsen's, that he had successfully cloned from sheep *blastocysts*, but this was in '89, I think, and the paper wasn't published until 2005, he presented it at the International Embryo Transfer Society. It seemed to me that it was almost certain that if he had cloned from blastocysts it would've been the *inner cell mass*, and of course around that time, the people who subsequently got Nobel Prizes for it,[4] were doing lots of exciting things with embryo stem cells in the mouse, so that idea of having embryo stem cells was very much in in the general discussion. And it seemed to me that if we could achieve two things, get embryo stem cells from livestock and do nuclear transfer from them, it would present great opportunities, either for multiplication or for genetic modification.

A *blastocyst* is an embryonic structure formed in early mammalian development. It consists of an outer layer of cells – the trophoblast – that surrounds the *inner cell mass* and a fluid-filled cavity known as the blastocoele.

We probably just succeeded in handing on the gene transfer technology to PPL Therapeutics, and certainly I was being cut free, having been obliged to be involved in the gene transfer project by Roger Land.[5] The deal was that once the transfer project was completed, or up and running, that I could go away and do my own thing again, and it seemed to me, having worked with John Clark and the other molecular biologists I learnt a little bit at least from them, that to try and create an opportunity for making precise change in livestock would be a useful

FIGURE 4, PREV. PAGE. Aerial image of Dryden farm, where Dolly was born, with the old Roslin Institute site in the backrground.

thing to do, a good thing to do. I'd never worked with Willadsen but I did know him, he succeeded me on a post in Cambridge when I came to Edinburgh, and I arranged to see him. By the time I heard about his work, which was done in Texas, he was in Calgary, and I happened to be going to Australia, later that year, and instead of going over Asia I went over America and on the way back called in to see Steen, and he was extremely generous with his ideas and his time, and confirmed that he had grown from cattle blastocysts, and described the technology to me. And so when I got back to Edinburgh, I discussed with Roger starting projects in this area, and I guess it was probably Roger who steered the thing towards the DTI [Department of Trade and Industry] funding initiative, rather than seeking BBSRC funding for it, and that was certainly the way we went.[6]

Lovell-Badge. So the main motivation was the pharming idea?

Wilmut. No, more general genetic modification.

Miguel García-Sancho. Could you describe the work you'd been doing in Edinburgh before, since you arrived from Cambridge in 1973?[7]

Wilmut. The work that we did before the involvement in the molecular biology project was to try to understand the causes of prenatal mortality, by investigating the relationship between embryos and the mother. We did quite a lot of work, mostly in sheep, one experiment in pigs, which went quite a long way to suggest that a significant amount of prenatal mortality reflects physiological variation, the embryo and uterus get out of step even in the same mother. Unfortunately, we worked ourselves into a corner, because if you say that there are maybe four or five causes of prenatal mortality, so that they might each cause four or five per cent loss, if you want to try to demonstrate that that physiological variation is causing that loss, you have to be able to hold the circumstances in which the animals are breeding, and their nutrition and everything, stable and uniform and consistent, so that you can look at the effect and variation of the one thing that you're looking at. And it's not possible, it's a Heisenberg uncertainty principle problem, so you can only suggest it as an interpretation.

Helen Sang. Can I just ask quickly? Was this all to do with farm animal reproduction or was it modelling for humans?

Wilmut. The suggestion would be that it applies to mammals. There's a prediction that the level of mortality in a particular species will be related to the biological costs of losing that foetus, and in humans, we're going to keep on trying anyway, no limit. So we can tolerate [prenatal mortality] in that sense – it's sad for people who can't conceive, I don't wish to treat it lightly and apologise if I cause offence – but it might seem predictable that we have a very high prenatal mortality. Species at the other end of the curve, like bison or seals, where they only get one shot – possibly one mating, certainly one reproductive cycle – the cost of losing that foetus would be very high, and the survival is higher. And rats, mice, sort of fit in the middle of that.

Alan Archibald. And pandas [*laughter*]?

Wilmut. What happened to me, I wasn't party to the original discussions which decided that the new molecular biology projects in the Institute were going to involve embryo manipulation, but the then director [Roger Land] approached me and essentially said that I was going to provide that expertise of recovering and transferring the embryos. I can't explain to you how angry I was [*laughter*]. I was one of the few PIs who was going to survive [the cuts at ABRO], a lot of the PIs were going to go, and I believe I owe that to Anne McLaren,[8] for which I'm eternally grateful. So I'd had a reasonable personal track record and was being told what to do. I was very angry.

García-Sancho. And this embryo transfer technique to surrogate mothers, did it receive commercial interest at the time?

Wilmut. The technique itself was nothing new, and it was probably started in a number of labs in the late 1940s and 1950s. Certainly the Cambridge [Animal Research Station] lab where I did my PhD had been involved; South Africa, New Zealand, Australia and the States, it would've been several labs around the world who developed this. So there was nothing innovative that we did in the procedure, but there was a company established here in Edinburgh, which offered the service of embryo transfer to farms. They did change the procedure. Ours was an intrusive surgical procedure, we just opened the abdomen, got the reproductive tract out. The commercial group used *laparoscopy* as a way of getting in.

Laparoscopy ('keyhole surgery') is a surgical procedure performed through a small incision with the aid of a camera.

Archibald. The commercial group were doing cattle, weren't they?

Wilmut. No, they were doing sheep as well.

García-Sancho. And what about Rick Lathe and John Clark, the molecular biologists with whom Ian Wilmut started collaborating in the mid-1980s? What were they doing before joining the pharming programme?

Archibald. Rick [Lathe]'s background was with Transgéne in Strasbourg, and they were in essence a small biotech company. The projects Rick did back then, when he worked with Transgéne, included developing an oral vaccine against rabies.

Alan Colman. They were dropping chicken heads [with the vaccine] from planes and helicopters over in rural France, trying to immunise the wild animals that might be carrying rabies.

Archibald. And John Clark, when he did his work in John Bishop's lab as a postdoc, arguably, wasn't doing genetic manipulation at all, he was looking at the expression of mouse [major] urinary proteins. And so the point of moving on to making transgenic mice wasn't until they established the lab at ABRO, and the plan was that they were going to make modified cells to test the constructs, then make modified mice to test the constructs further, and then make the sheep. And we made the sheep before we had success in mice, if I remember correctly.

Colman. It's hard to know if what you're going to say is in or out of the immediate context, but it follows on from what Ian was saying, if I could just give the history of PPL coming to Roslin, as it sets the context for the type of relationship we had. I'd been an academic, I'd done my PhD with John Gurdon,[9] and one of the things I did as an academic was to inject messenger RNA into frog eggs. I was making mammalian *interferon* from frog eggs, and someone said 'well, why don't you start a company with you injecting frog eggs with interferon RNA?' and this obviously wasn't a feasible proposition, but that seeded in me the idea of going to chicken eggs, and you [Robin Lovell-Badge], I think, shortly after this wrote a little piece in *Nature* about pharming in chicken eggs, that is, making foreign proteins in chicken eggs – I think it was chickens?

Interferon is a protein released by animal cells in response to a virus, which has the property of inhibiting virus replication. It is used as a pharmaceutical.

Lovell-Badge. No, the first transgenic farm animals were being published, in *Nature*, by Ralph Brinster and people, and I was asked to write a 'News and Views' on that, on the first transgenic sheep, pigs and rabbits.[10]

Colman. Well, the fact was we thought eggs would be a good proposition because laid eggs present a sterile environment. We were worried about the use of farm animals for the same purpose because of, you know, the dirty conditions they live under. At the time I was consulting for Prudential, an insurance company, earning a little bit of pin money, and we were looking for somewhere and someone who could do the work. One way or another, this search led us up to Roslin and Helen Sang. We set up a project where I was a sort of project monitor, to make transgenic chickens, and it was by that contact with the Roslin Institute, though it wasn't called that at the time, that I became aware of Clark's and your [Ian Wilmut's] work on the production of human proteins in the milk of transgenic sheep. I had no idea about this work when we started the transgenic

FIGURE 5. Tracy, born in 1990, was the first transgenic sheep made at Roslin to produce large amounts of pharmaceuticals in her milk. © Roslin Institute.

chicken project. As a result of this realisation, we (myself and Prudential) decided to sponsor work on the farm animals, and ultimately, a company was started. There were problems with starting a company at that time with a government-run institute because although nowadays what would happen is that the Institute would start the company, then it was expected that outsiders would fund it. I recollect that government rules prevented that type of initiative, so you guys couldn't start your own company, so we licensed the technology and started a company. The company subsequently became known as PPL but originally it was known as Caledonian Transgenics, a very parochial name. That's where our involvement in the pharming started.

Now to return to the subject of making proteins in the milk of transgenic animals, we wanted, ultimately, a more reliable way of making transgenic farm animals because the method of *DNA microinjection* of fertilised embryos, which you, Mr Chairman [Robin Lovell-Badge], summarised in your 'News and Views' article, was very inefficient, with only three to five percent of the animals born to the microinjection technique being transgenic. In mice, it was possible to use genetically-manipulated embryonic stem cells to make transgenic mice. Many groups including ours at PPL were looking for farm animal embryonic stem cells for the same purpose, so we were looking round the world for sources of such embryonic stem cells. Our search failed and it was really when we heard of, I think it was Jim McWhir and Ian [Wilmut]'s nuclear transfer work, that we were alerted to possibly a new way of thinking about making transgenic animals, and also making precise changes in the genome as had been done in mice.

DNA microinjection or *pronuclear microinjection* is the earliest and most common method for making transgenic mammals. In this procedure, solution of DNA of interest is injected directly into a pronucleus of a one-cell emrbyo (zygote) under a microscope using an instrument called a micromanipulator. The pronuclei are the two nucleui contributed by the egg and sperm – they do not merge until the first cell division of the embryo.

Archibald. Going back to the pharming, of course the whole idea of trying to make valuable therapeutic proteins in farm mammals, or animals, went back to

the first efforts in terms of moving up to genetic modification at what was then IAPGR. When Rick Lathe[11] came on board as head of molecular biology, we touted around the idea, round the big pharmaceutical companies, we touted it to the British Technology Group, all of them were saying, 'This isn't going to fly, this isn't going to work.' Where arguably Rick and John Bishop[12] started from was the closing paragraph of Brinster and Palmiter's paper on transgenic mouse[13] – the giant mouse – which said, 'it has not escaped our notice that these animals have got huge levels of circulating growth hormone', and so of course the USDA [United States Department of Agriculture] Beltsville laboratory went along the route of trying to produce transgenic pigs, and after looking at other bits of the second Brinster and Palmiter paper[14] – and by the way these animals have got arthritis, this, that, the next thing, and fertility problems – we picked up on the almost throw away remark, the high levels of growth hormone, and we said, why don't we try making something more interesting than growth hormone, and do it in sheep?

Lovell-Badge. So then the first products that you tried to make were … *Alpha-1-antitripsin* was one, but there were several.

Alpha-1-antitrypsin is a protein used in the treatment of emphysema and cystic fibrosis. It was a key product for PPL.

Colman. Correct, we were quite lucky in that the first sheep we ever made in the collaboration with Roslin, called Tracy the Transgenic [born in 1990], made an extraordinary amount of human alpha-1-antitrypsin in her milk, which caught the attention of many people when it was published in *Nature Biotechnology* (*Fig. 5*).[15] The company attracted a lot of inward funding from that point, because of that one demonstration.

Lovell-Badge. I think there were attempts to make mice with the alpha-1-antitrypsin expressed in the milk, but the mice we made in London using just the human gene with its own regulatory sequences were making more.[16]

Archibald. Well, the alpha-1-antitrypsin construct that PPL injected produced Tracy.

Lovell-Badge. That was tried in mice first, though.

Bruce Whitelaw. Yes, it was.

Archibald. Yes, they put that into mice, so that was the *beta-lactoglobulin promoter* sequence that John [Clark] had isolated. We cloned the beta-lactoglobulin gene from sheep, and the alpha-1-antitrypsin one was the genomic construct that I'd got from Gavin Kelsey, because I'd been in some meeting in London where Gavin had described his work with putting that into mice.

A *promoter* is a DNA sequence immediately upstream of a gene that controls its expression, i.e. how much RNA and then protein is made. The *beta-lactoglobulin promoter* was isolated from a milk gene and targets gene expression to the mammary tissue and milk.

Lovell-Badge. It was the human gene?

Archibald. Yes, and the human alpha-1-antitrypsin promoter, and that worked very well as far as I can remember. Okay, this is a simple cut and paste job, and it was.

Schnieke. It works very poorly with the *cDNA*.

cDNA, or *complementary DNA*, is reverse-engineered from messenger RNA and not isolated from the genome, and thus does not carry non-coding DNA elements.

Archibald. Yes, we had a whole string of constructs.

Whitelaw. We made a series of constructs, we called them A, B, C and D. It was B that you ran with, the one Alan's talking about. We made various versions of the C and A ones carrying alpha-1 or *Factor IX,* and we put them into mice, and most of those were poor, to say the very least.

Factor IX is one of the blood clotting proteins. Its deficiency causes haemophilia B. It was another pharmaceutical that PPL pursued.

Colman. Do you remember where you got it from?

Whitelaw. It was from Gavin Kelsey, we got the alpha-1 from Gavin Kelsey.

Colman. And where did he get it from?

Whitelaw. He cloned it, did he not clone it?

Lovell-Badge. He was in Sue Povey's lab, she had it.

Colman. Where did she get it from?

Lovell-Badge. They *cloned* it [*laughter*]!

Here, *cloning* refers to isolating and amplifying genes.

Archibald. I remember, for comparative purposes I got hold of some of Gavin's

mice. I must've been in London and I brought them back to Edinburgh on the train, and it'd been a long series of meetings I'd had in London, so I was really tired and I kept falling asleep and having nightmares about the train derailing and these mice escaping [*laughter*].

Harry Griffin. It might be useful just to reprise exactly how Tracy was made.

Colman. Okay, this involved a microinjection of a human gene into a fertilised egg, into one of the two *pronuclei*. The human gene was linked at the DNA level to a sheep milk protein promoter called beta-lactoglobulin which had been isolated by Clark, and this was the driver if you like, the gene switch which made the human protein production almost exclusively in the mammary gland and therefore it went into the milk. We'd hoped, as had they [IAPGR scientists in Edinburgh], that ethically this approach would find some favour with the movements that were set against the use of animals in research – and of course, Roslin was a target for the animal liberation movement, and there had been an arson attack at some earlier date.[17] As a result of public concerns, when Tracy and animals like Tracy were made, we were told by the government to make sure that psychologically these animals were alright. We had already established that physically the animals were unaffected by the *transgene*. Subsequently, a paper was published describing 20 transgenic sheep and 20 related siblings that didn't have the gene, sitting in a field, and their behaviour over a six week period was noted by animal psychologists, with the conclusion that the transgenic sheep were no different in behaviour.[18] As I say there was a lot of attention, a lot of concern about transgenesis in large animals in case it created monsters and all those sort of things. So we were always treading a line, as a company, worried about the public acceptability of what we were doing.

A *pronculeus* is a nucleus in a one-cell embryo (fertilised egg, or zygote) from either the egg or sperm – the two do not merge until the first cell division of the embryo.

A *transgene* is a foreign gene injected into the zygote to make a transgenic animal

Lovell-Badge. So, was the idea of making proteins like alpha-1-antitrypsin driven by being able to make large amounts of protein at a relatively low cost?

Colman. Yes, we were trying to meet an unmet clinical need. This particular protein is needed by congenital emphysema sufferers and they need infusions of four grams a week. They get the material at the moment (and at that time) from blood supplies, but there wasn't enough blood available to satisfy the needs of all the sufferers.

Lovell-Badge. It was also not so long after HIV/AIDS and the whole idea of contaminated blood products. That was in the mid 80s, people were looking at ways of making clotting factors and other valuable proteins that you could only get from [blood].

Archibald. The two proteins we had, if you like, in the portfolio initially were Factor IX, a blood-clotting one, where the issue with producing that, using biotechnology rather than isolating from blood, was the complex *post-translational modification*. With alpha-1-antitrypsin, it was the volume issue, in either animal cells, or trying to produce it in bacteria or yeast. You could not, if you had harvested all the blood products of the blood transfusion service, have enough to treat people with emphysema. Whereas with Factor IX, it's modest amounts and a relatively small population to treat, alpha-1-antitrypsin was much more challenging in terms of trying to meet that market, well market is not really the right word for it …

Post-translational modifications are chemical changes to proteins that happen after they have been synthesised from an RNA template. These differ between bacteria and eukaryotes, making animal systems more attrative for pharmaceutical production of complex porteins.

Lovell-Badge. Does anyone want to add on to this? So the whole concept of pharming, were there several places around the world doing it?

Colman. Yes, as I said, we thought we had a lead in this area when we began the transgenic egg project, but truthfully it was very difficult to make transgenic eggs, terribly difficult.

Sang. Yes, we didn't really have the technology, because manipulating a hen's egg is much different from manipulating a sheep egg, and so it took us too long

to get to the point where we could actually start making proteins in eggs. And the work on milk and sheep took off, and I think working with sheep, part of that was that sheep were much cheaper, is that correct? Rather than working in cows, for example?

Wilmut. I think it was more practical at the stage of producing the animals, actually handling the animals and recovering the embryos for transfer – plus we had the experience with sheep. And it was very likely that if we made something work in sheep it would also work in cows, which is true, so you could just apply a proven method in cattle.

Archibald. And of course, the company that succeeded at the end of the day at getting a product into use [GTC Biotherapeutics], as far as I recall, produced it in goats.[19] Cattle are obviously a damn sight more expensive, you're dealing with 100s of eggs in order to get a successful one.

Colman. I think another company got milk products approved for sale, human milk products out of rabbit milk. The company was called Pharming, in Holland, and it was our biggest competitor at the time. They also made Herman the Bull.[20]

John Bracken. A question for Alan [Colman]. You were so successful with Tracy and the amount of milk she produced with the human protein. Did you ever repeat that to the same degree, or was that the best you ever managed to achieve in the production of protein?

Colman. No, for the alpha-1-antitrypsin she was the best ever, but we subsequently made a sheep expressing in its milk 70 g/litre BSSL [Bile Salt Stimulated Lipase, a breast milk enzyme involved in fatty acid metabolism]. We had licensed the gene from a group in Sweden, however, no further development of this project occurred.

Sang. You couldn't milk them [*laughter*].

Bracken. Was that microinjection again?

Colman. That was microinjection, yes … So it could be a wonderful technology. I mean, it turned out that goats were probably a better choice as a medium-size animal, because the average milk-yield of a goat is a lot better than that from sheep, but there you go.

Bill Ritchie. I think that you've got to remember that at that time all the oocytes, the embryos, were actually recovered by surgical methods, so that made it much more difficult for you to actually use something like cattle. Probably sheep were the best animal to use for any of these experiments.

Wilmut. I think that's a good point, I mean, by now, with the proved technologies for in vitro maturation and fertilisation in embryo culture, you probably could make it work in cattle, it would be reasonably economical.

Colman. I think when we did cattle in the US and we used in vitro maturation from abattoir cow materials to get the eggs, we never used live donors at all, but that was some years after of course …

Ritchie. I think the technology developed, but when we started doing these experiments all the oocytes or embryos we were using were recovered by surgical means. That was a huge amount of work, and a huge amount of people were actually involved in it, because all these animals *superovulated*, so that involved farm staff as well, to actually superovulate these animals.

Superovulation is a hormonally-induced procedure that results in female animals releasing more eggs that usual, so that more embryos can be manipulated or transfered.

Lovell-Badge. Any specific questions? Miguel [García-Sancho], you did ask me, is pharming an appropriate term?

García-Sancho. Yes, one thing we were wondering is what word you used at the time to designate that line of research on genetically modifying animals for a particular purpose. I wasn't sure if the word pharming arose after the actual genetic modification efforts at Roslin. How did you designate that research programme at the time, which term did you use?

Lovell-Badge. The word pharming had come earlier, much earlier, it was being used in other systems too, in plants.[21]

Jim McWhir. While it's true that PPL's interest was, I think exclusively, in biopharming, we should point out that those of us at Roslin probably had a wider range of interests. We were interested in the possibility of making farm animal models of human disease, for example, and doing that using *gene targeting* technology. There were also those of us who were interested in being able to use a *somatic cell* intermediary so that we could assess levels of gene expression prior to going to the expense of making an animal. At that time I'd just got my first Medical Research Council grant, to engineer mouse embryonic stem cells and look at the possibility of deriving therapeutically relevant somatic stem cells in vitro, and we were interested in doing that ultimately in farm animals. Have I forgotten anything, Ian [Wilmut]?

Gene targeting is a precise way of genetic modification, where a gene can be inserted, changed or replaced in a specific location in the genome. It is normally achieved by using stem cells that can be modified and selected in culture.

A *somatic cell* is a non-reproductive ordinary cell of the body, i. e. not a germ or stem cell.

Lovell-Badge. There were other areas of potential interest, so obviously disease resistance in farm animals. Still goes on today.

McWhir. And then, of course, reproductive cloning of elite animals, but I think that was probably of the least interest.

Wilmut. I don't think anyone's mentioned animals as donors of organs.

Angelika Schnieke. Within PPL we had two areas of interest. We had some projects where we wanted to replace the ovine or bovine gene with a human gene. I think we had two or three different projects, for which we needed the *homologous recombination* (i.e. gene targeting) technologies. We needed to have some types of cells, either embryonic stem cells, or some kind of substitute, where we could carry out homologous recombination. And then we also started in the area of *xenotransplantation*, and again, we needed to be able to manipulate endogenous genes, to be able to carry on in this area. At the time we worked a lot together with Jim in the area of stem cells and homologous recombination, and through him we also became involved in the whole Dolly project.

Homologous recombination is a process by which two similar or identical parts of DNA molecules can swap parts. It is a key mechanism for gene targeting.

Xenotransplantation is the transplantation of tissues or organs across species.

García-Sancho. You have also mentioned a very interesting collaboration between Alan Colman and Helen Sang, making transgenic eggs. There was another important line of research at what was then the Poultry Research Centre on making transgenic chickens. I was wondering whether you could discuss if there were connections between this programme and the one on sheep and any other animal you might have explored at the time.

Sang. If we go back even further in history, it was when molecular biology was really taking off in bioscience and in Edinburgh in particular, and at that time there were two institutes. There was the Animal Breeding Research Organisation [ABRO], which was at King's Buildings [in South Edinburgh, next to] the University of Edinburgh [buildings] and the Poultry Research Centre that was in Roslin village. There were new appointments made, and people began to get involved in molecular biology at both sites, and then we were put together as part of the Institute of Animal Physiology and Genetics Research [IAPGR], and the people from ABRO were moved out to the Roslin site. We had this going on, applying molecular biology as a research tool in general, but also trying to develop methods for genetic modification in chickens, at the Poultry Research Centre, and in farm animal species at ABRO. And then we all were merged together and I would say, we had a slightly uncomfortable relationship, really. Bringing the two institutes together wasn't particularly smooth.

Lovell-Badge. In what context [did you attempt to apply molecular biology] other than mammals and chickens?

Sang. I think it took a while, and certainly I used to go to meetings with Grahame Bulfield, and Clark, and Lathe, and Paul Simons, and – I don't think you were at those, Alan [Archibald]?

Archibald. I'll tell you in a minute.

Lovell-Badge. But was it also in the context of a general, more quantitative genetics of traits [in animals]?

Sang. The Poultry Research Centre had very little [quantitative genetics], and the real quantitative genetics was a strength of ABRO, and that's an ongoing strength at the Roslin Institute

Griffin. Your major challenge in manipulation was that by the time the embryo was accessible, it was 10,000 or 20,000 cells

They stated that PPL were going to make lots of money, so there should be a sheep called Bolly, after a well-known champagne

ALAN COLMAN

Sang. 60,000, yeah.

García-Sancho. Alan Archibald, were you also involved in these meetings?

Archibald. I've spent my entire life in meetings since 1981. The merger of ABRO and the Poultry Research Centre was kind of the next phase after the Thatcher government tried to effectively close it [ABRO], and so moving into molecular biology and into the kind of work Ian [Wilmut] was already involved in. But under Roger Land's leadership we possibly took a greater emphasis in terms of what we were doing, in assisted reproductive technologies, plus moving into molecular biology. And there were two possibilities in molecular biology. One was to get into what in essence was the foothills of genomics research, which was my own particular interest, but Roger was putting more emphasis on genetic modification, which is, in essence, where the pharm[ing] project grew out of. It was that particular idea that you should use molecular biology to change animals, rather than to try and work out what genes are controlling the traits. We were running those two strands in parallel, and the genomics bit took off five years later.

Wilmut. Yes, reproductive manipulation techniques were going on under John King,[22] that's the reason, in a sense, why I was recruited to ABRO [in 1973], to bring in those sort of technologies, not for the purpose that they were actually used for, but more for use in the Hereford context.[23]

Archibald. So there was a kind of coming together of the assisted reproductive technologies and quantitative genetics with the sort of thing Charlie Smith was doing in terms of *multiple ovulation embryo transfers*.[24]

Wilmut. That was the objective. It never really happened, I think, because the facilities which were ultimately made available took a long time to come, we were doing other things.

Sang. I think it is important to know that there were these different streams of activity going on with very different expertise, and that's what was pulled together in bringing the whole transgenics and then cloning.

Multiple ovulatuion embryo transfer (MOET) is an enhanced method for transplanting embryos between animals. It relies on hormonally-induced *superovulation*, i. e. making a female produce more eggs than she normally would.

2. Dolly and her flock

Lovell-Badge. The next topic I was going to raise is the relationship between Dolly and the sheep that preceded and succeeded her. My little list seems to include Tracy (we've talked about her already), Morag and Megan, Dolly, Holly, Ollie, Molly, Polly, and then Cupid and Diana. So, no Folly?

Colman. And no Bolly. They stated that PPL were going to make lots of money, so there should be a sheep called Bolly, after a well-known champagne [Bollinger]. And the name Trolley was suggested from the saying 'they're off their trolley' for doing this sort of thing – so lots of humour in the press!

Lovell-Badge. Obviously, Morag and Megan were the first cloned sheep (*Fig. 6*).

Wilmut. Yes, once we got the project started, the key to it was Keith [Campbell], really, who investigated what happened after you did nuclear transfer, how to optimise the process. He did a PhD in Sussex.

Sang. With Chris Ford, I think.

Colman. There were frogs involved in some of the work he did.

Wilmut. He was looking for *MPF [maturation promoting factor]*, looking to purify and identify the components of MPF. It was not surprising in a way that if he was working on his own he didn't make much progress, but in order to make progress with the cloning, we needed to know about that activity, so as somebody who had thought a lot about the role of these proteins in cell cycle, knew one or two key assays to follow the activities, he was perfectly placed to analyse this.

The *maurations promotiong factor (MPF)* is a protein complex that initites cell division.

Lovell-Badge. So, what was the reason for going for *oocytes* rather than fertilised eggs, which had been tried in the mouse?

Wilmut. There were one or two people doing mouse work who were beginning to use oocytes, people in Japan. I can't remember how this would've fitted in with what we did, but you'd fairly quickly have had subjective assessments that oocytes were giving you better reprogramming. But they wouldn't have a high level of MPF activity, I don't know the answer as to why they didn't. I guess it could have been because when we started using oocytes we wouldn't have had the profile of MPF activity, it would have taken a few months of research activity to do that.

Lovell-Badge. And then there were various tricks employed like *blocking mitosis*?

Wilmut. Yeah, there's a limit to how long you can hold cells before you poison them, and it's probably not a very accurate process. The cells of the early embryo don't have the normal *checkpoints*, so it's actually biologically not easy to stop them. So, really, if you're going to use cells from early embryos you'd do much better to *pre-activate* the oocytes and then just transfer a donor nucleus in, at any stage of the cell cycle – you know, it's practical. That's what we did with the sheep that I suspect Megan and Morag came from.

Mitosis – the cell division of the vast majority of cells except the germ cells – can be blocked in its various phases using chemicals. *Mitotic checkpoints* are control mechanisms which ensure proper cell division.

Oocytes used as recipients in nuclear transfer respond better when they have been *pre-activated* by chemical simulation of maturation.

McWhir. They came from the TNT [*totipotent* for nuclear transfer] cells, which was the cell line that we isolated.

Wilmut. Yes, I remember that, but I think Keith [Campbell] checked and showed that they would stop [the developmental cycle], and also start again.

Lovell-Badge. Why was that particular cell line chosen?

A *totipotent* cell is capable of giving rise to any cell type.

Wilmut. Jim [McWhir] had been trying for a number of years to get stem cell lines going out, and the way I remember the description is that you didn't get a massive, bulging out of a different cell type, the ones we were interested in sort of simply disappeared. Would you say that's a naïve but fair description?

McWhir. We had isolated something, which probably technically wasn't a cell line, it depends on how you define a cell line, and if you define a cell line as something that's adapted to perpetual culture, it wasn't, but we could keep these cells going for 15 or 20 *passages*, which was probably enough to do genetic modification. So although we had no evidence that they had the properties of embryonic stem cells, they did have some of the properties of cells that we could genetically modify, and so they were interesting candidates for nuclear transfer for that reason.

Passaging involves transferring some or all cells from a culture to fresh growth medium, in order to prolong the life of the cultured cells.

Wilmut. The idea of that experiment was to do nuclear transfer from this population, to see whether the ability to support development stayed the same or whether it decayed and, if so, over what time period.

Colman. Could I just clarify, because this is new to me, so the nuclear transfer was secondary, it was just a test of the totipotency of those nuclei?

Wilmut. Yes, and it didn't come out very cleanly, that's the problem. It did drop [the cell's developmental capacity], but it was sort of a dithering line.

McWhir. I think probably different people have slightly different interests for obvious reasons, so correct me if I'm wrong, Ian, but I think you were interested in evaluating the differences between the behaviour of cells of different origins in nuclear transfer. And I was primarily interested in the possibility that the TNT

FIGURE 6. Megan and Morag, born at Roslin in 1995, were the first sheep cloned from cultured embryonic cells. © Roslin Institute.

cells might be useful for doing the kinds of things that we wanted to do down the line, so Ian's interest was possibly slightly more basic than mine, mine was a bit more utilitarian. Would that be a fair summation?

Wilmut. Yes.

Schnieke. I think that was also the point where PPL became interested. I can remember we once sat together at lunch: Alex Kind, who was doing cell culture and stem cell work at PPL, you [Jim McWhir] and I. This was the time of the Megan and Morag pregnancies, I think it was still early pregnancies, and Jim was telling us about his cells, that he can culture them for a while, and that they had given rise to these pregnancies. And that's when we discussed that this could also be of interest for PPL because we wanted to carry out genetic manipulation. If we had cells in culture that we could manipulate, and if they could then give rise to pregnancies this would be an ideal project. That's when you [Jim McWhir] suggested maybe I do a PhD, and I went to Alan [Colman] and asked for financial support for this [*laughter*], and that's when the next part of the project started, when the next animals were generated.

McWhir. Well, I think that illustrates the value of this exercise because I had actually forgotten that, but I now remember it.

Schnieke. It was in the canteen at the Roslin Institute where we were sitting at lunchtime – best meetings ever.

Colman. But Megan and Morag is really the most important of the publications, not the Dolly one, which was where the technology was further validated.

Ritchie. That's what Keith [Campbell] would always say: that was the most important experiment, Morag and Megan, and Dolly was a consequence but not the real breakthrough.

Wilmut. There's been an awful lot of discussion about the authorship of these papers.[25] I'm interested to hear you say what you've just said. I was going to be the first author of the Megan and Morag paper, but because he [Campbell] put the *G_0 component* in, I suggested he should have it and I would have the next one, and that's why I ended up with Dolly [*laughter*].

Sang. Maybe Harry [Griffin] can remember – Megan and Morag's was a paper in *Nature*, wasn't it? And there was a lot of interest, which hadn't really been anticipated, so when the Dolly paper was coming along, Harry in particular sort of made sure that people were ready in case there was interest, but there was still the feeling that maybe it wasn't as significant as Megan and Morag.

That's what Keith [Campbell] would always say: that was the most important experiment, Morag and Megan, and Dolly was a consequence but not the real breakthrough.

BILL RITCHIE

In the *G_0 phase* of the cell cycle, the cell is inactive or quiescent and does not undergo division. Forcing cultured adult cells into this phase was thought to be the key to making Dolly possible.

Griffin. I think the media interest for Megan and Morag lasted about seven days, so there was interest, but nothing like when Dolly was born, or at least announced.

Bruce Whitelaw. There was an unfortunate reason for that cut-off of interest – Dunblane [school massacre].[26]

Colman. Is that right?

Whitelaw. Dunblane happened about six days after we announced [Megan and Morag]. But even with that, the interest was in a different league when it came to Dolly.

Griffin. Maybe it's appropriate, given we're discussing the timeframe here. When was the patent taken out?

Wilmut. 1995

Griffin. And what did it cover?

Wilmut. Everything we could think of.

Griffin. Because it was taken out after Megan and Morag were born.

Colman. Yes, but before [the research] was published. We poured over the patent. There was one unfortunate aspect of the patent specification. It insisted, I think, that the use of G_0 cells to provide nuclei was the secret to success, and people at the time (although we challenged it successfully later) argued that if you used another stage of the cell cycle other than G_0, it wasn't conflicting with this patent. We suggested that the patent should have used the term 'preferably G_0.' The use of 'preferably' is a common patenting device to improve the breadth of the patent claims.

Wilmut. Even preferably is a bit strong. It would have been better still if it had been, in laymen's English it would be awaiting DNA replication, for example, in G_1 or G_0.

In the *G_1 phase* of the cell cycle, the cell prepares for DNA replication by synthesising RNA and proteins.

Lovell-Badge. And was the patent taken out by Roslin?

Colman. Oh yes, they made the technology available for licensing.

Lovell-Badge. So there was some media interest. I remember a little bit myself, but it wasn't huge amounts.

Griffin. No, I wasn't involved in handling that, probably, Ian, you were.

Lovell-Badge. But there was a lot of scientific interest, I'm assuming. Because they would've been the first mammal clones.

Wilmut. First from a cultured cell line was, I think, how we described it.

Schnieke. But I think the emphasis was probably still slightly different in the publication, it wasn't so much that it was a differentiated cell, it was proposing that it might be a stem cell.

Lovell-Badge. Embryonic, yes.

Schnieke. Yes.

Lovell-Badge. I remember there was a lot of discussion about whether it was still embryonic or not.

Colman. And I think I'm right in saying that you and Keith were quite concerned after the publication, because there'd been a lot of losses, in pregnancy and everything, and you were worried about the knock on effect of making unhealthy animals, and things like that. Quite concerned, weren't you?

Wilmut. It was when the effects on birth weight first became apparent as well.

McWhir. Can I return briefly to the question of which was the more important paper [*laughter*]? Because I think I have a slightly different perspective, although I had more involvement in the Morag and Megan paper, so it would be very congenial to me to think it was important. But my view of the history of the cloning technology is that previously, there was a received wisdom that cloning worked for very early pre-implantation embryo-derived cells, and as you took those cells from progressively later developmental stages, the efficiency rapidly plummeted and was effectively zero after the blastocyst stage. And so, the conventional wisdom was that there was an irreversible differentiational commitment, and that's why so many people disbelieved, initially, the Dolly result. So in my mind, when the Morag and Megan paper came out, possibly because I didn't have the insight that Ian and Keith had into the techniques that had been used in developing the cloning, in my mind it was still very much up in the air, whether or not that success was a property of the cell of the embryo-derived cell line, or whether it was a consequence of the modifications that Keith and Ian had introduced into the techniques. So the way I think of this is that the Dolly paper showed unequivocally that there was nothing special about the TNT cells, and that you could take probably any differentiated cell and completely reprogramme it. Convince me I'm wrong, but I would say that the Dolly paper was more important.

Dolly [...] showed unequivocally that there was nothing special about the TNT cells, and that you could take probably any differentiated cell and completely reprogramme it. Convince me I'm wrong, but I would say that the Dolly paper was more important.
JIM McWHIR

Wilmut. Can I ask a nasty question? Did Shinya Yamanaka or anybody else rush off to produce *iPS cells* after Megan and Morag?

Induced pluripotent stem cells (iPS cells) are a type of a stem cell that can be generated by manipulating differentiated adult cells.

Colman. No, I think people were inspired by the Dolly experiment. I think what I was referring to was the technical tour de force, which was in the first paper, and the second paper just used the same technology, but you have a point.

Schnieke. It proved a dogma.

Ritchie. Yeah, I think that's right, in that the techniques that were used, in the actual cloning itself, were so much different to what had gone before. [With our technique] you had the potential of genetically modifying those [cells], whereas you didn't have that in the previous technique [used by Willadsen], where you were taking an embryo which has 16 cells.

Lovell-Badge. Also, in terms of frog experiments, you could clone from a larval stage of a frog, but you couldn't do it from an adult.

Colman. That's correct, and you still can't. As we'll discuss later, when we were discussing the idea of putting [adult] mammary cells in, which was late in the day, I didn't really feel it would work at all because of the John Gurdon experiments.

Schnieke. Exactly.

Colman. I just didn't think it would work and some of the others didn't think it would work. Some people didn't say anything but I think no one absolutely thought it would work.

Wilmut. Keith [Campbell], probably.

Colman. I don't think so … I spoke to Keith, he didn't, he was neutral.

Schnieke. I think it took a long time convincing Alan [Colman] to support the project using the cells that then gave rise to Dolly.

Wilmut. You [Alan Colman] made a wager. Do you remember making a wager? Said you'd eat your hat [*laughter*]. He hasn't, but we could provide one, I'm sure.

Schnieke. I think you also made a comment to me, 'it probably won't work.' And I think we were very lucky.

Colman. Well, when we come to talk about the experiments that led to Dolly, it'll be very interesting to hear what was going through the thought processes of the Roslin people, because this collaboration was an example of a commercial organisation interacting with an academic [institution], where the commercial organisation, because the academic did a lot of the technical work, had to share with them exactly what they were using. In contrast, there was a part of the Dolly series of experiments which we never knew about until there were births. We never knew about the use of *foetal fibroblasts*, so the secrecy was rather one-sided here but that was the way it was.

Fibroblasts are the most common cells in the connective tissue. Sheep *foetal fibroblasts* were among the cell lines used in the Roslin cloning experiments.

Lovell-Badge. What happened to Megan and Morag?

Wilmut. They're in a museum, The National [Museum of Scotland].

Ritchie. Only one of them is.

Wilmut. Which one?

Colman. Megan.

Wilmut. Yeah, you're right, because the other one lived for a much longer time, didn't she?

Ritchie. Yeah, she did.

Lovell-Badge. What's the reason?

Ritchie. Well, the other one lived for fourteen years.

Wilmut. Really?

Ritchie. It was put down because of extreme old age. It became a pensioner.

Lovell-Badge. So then, Dolly. Let's explore what you were talking about, the choice of cell lines.

Colman. I think we can all contribute to this. The collective memories might be different and it'll be interesting to see what we did. We wanted to follow on from the work you were describing, Jim [McWhir], and make lines, embryonic-derived lines, which were qualified so that they could be used in our own facility. We had a very clean facility, because at the end of the day, we were trying to make drugs for human use, we wanted to get some of our own embryonic cell lines and we wanted to validate them by nuclear transfer, and wanted to collaborate with Roslin, because they were the people who could do that. We couldn't perform the technique by ourselves at that time. We made four – Angelika can correct me – four cell lines, different lines, and we wanted to use two thirds of the sheep that we'd communally bought – Roslin actually bought them and paid for them, but we had agreed to pay I think for two thirds of the overall project. We wanted to just validate the cell lines by getting live births from each of them. Had that occurred, we'd have four different lines we could play with for genetic engineering.

Because we were a commercial organisation we wanted to have a contract, and we wanted to be able to license the technology that Roslin had patented so that we could use it in the future. We wanted two types of licences: one for making proteins in milk, which is where we already had expertise, and also organ transplantation, to use the technology to *knock out* genes and modify pig organs, in fact. We hit a block there, because Roslin, probably rightly, didn't want to license the xenotransplantation uses to us [PPL] because we had no track record at all in this area, so they wanted to license it to an organisation that might be better placed to take advantage [of the patents], so they didn't want to give us a licence

Knocking out a gene means inactivating it using gene targeting techniques. In xenotransplantation, this might be used to inactivate immune genes.

for that. There were interminable negotiations going on, and meanwhile, the sheep season – because these animals are seasonal breeders – was going to close, and that would mean if we did these experiments together we'd have to wait another year to actually get started. Neither side wanted to do that, so I made the decision to go ahead with this project, even though we didn't have a signed contract or licence. We just had a gentleman's honour agreement, and I have to say that, at the end of the day, Roslin completely honoured that, but at the time we didn't have a contract.

We set up a project management group of myself, Angelika, Alex Kind from PPL and I think it was Jim [McWhir], Keith [Campbell] and Ian [Wilmut] from Roslin. We'd have monthly meetings, I think – I can't remember the exact frequency of the meetings – just to monitor progress, and I think what happened was that two of our four lines, I think it was two, fell by the wayside. They were *karyotypically* abnormal, therefore you would never contemplate using them for nuclear transfer, and we didn't have any other lines available, embryonic [lines]. And I think that's when Angelika piped up, 'Well, we've got this mammary cell line which we've had frozen for two years,' and we developed that mammary cell line to do what you mentioned earlier, we wanted a quick assay for transgenic constructs to see if they were going to go [work] in sheep. The idea was you put the [DNA] construct into a mammary gland cell in culture and see how much milk protein it produces. Then you can refine your construct and introduce it, by the conventional microinjection procedure, into an animal. But the problem was, when you take these mammary cells into culture, they dedifferentiate very rapidly and they are pretty useless as mammary cells, so we just froze them away but you [Angelika Schnieke] had karyotyped them and shown them …

Karyotypic abnormality suggests large-scale chromosomal mutations, such as rearrangements or deletions of chromosome parts. A *karyotype* is an organized profile of an organism's chromosomes, which includes number and microscopic appearance.

Schnieke. I remember it was in the early days when Alex [Kind] was still working on the mammary cells, that we had the idea that if you could actually differentiate them, use them to test your transgene of choice and show that the transgenes are expressed, that you could then use the same cells to make the sheep via nuclear transfer. That's when we first suggested the idea, but it was seen as much too risky. We carried on trying to isolate the embryonic cells, and then we had a problem, like you said, I think one or two isolates got contaminated and fell by the wayside, and we spoke then again that maybe now we should try out these mammary cells. If the whole project would work we could actually do a readout and then produce the animal. In the end, we got the animal, the readout didn't work [*laughter*].

Colman. Yeah, because of my own background working with John Gurdon, I knew a lot about frog nuclear transfer, and I didn't think it would work, but we didn't have any other validated cell lines and we had all these sheep that had been purchased, so we went ahead.

Griffin. And by chance those cells came from another animal research institute.

Schnieke. That's right, it was in collaboration with the Hannah Institute.

Griffin. Which was quite useful, a little later on.

Colman. It was, yes, when we were challenged.

Schnieke. And the cells were from an old sheep, and that also made it very interesting to see if those cells could still go through the nuclear transfer.

Ritchie. I think we were very lucky to actually get animals from these cells at all, because we did the experiments the last two or three weeks of the sheep breeding season, and we actually increased our nuclear transfer days. We used to do two a week, and we actually increased these to three days a week so we actually did it.

Sang. So were the cells from an adult female?

Colman. Yes, six years old.

Sang. Six years old? Wow.

FIGURE 7. On the day of the event, Elder Room, Old College, University of Edinburgh. *Left to right*, Jim McWhir, Alan Colman, Angelika Schnieke, Bill Ritchie, Robin Lovell-Badge. © Dmitriy Myelnikov

Colman. We were totally unaware that Keith [Campbell] and Ian [Wilmut] were using foetal fibroblasts in their part of the project.

Wilmut. That surprises me, I must say.

Colman. Yeah, we just didn't know.

Schnieke. I mean, you can probably confirm it, Keith would not say what exactly he was doing [*laughter*]. Would not want us to know how he treated the cells. The different cells which we isolated, whether it was the embryonic cells or the mammary cells, we had to hand them over to him for his final treatment, and he would not allow us to know what this was.

McWhir. Well I feel very honoured, then, because he told me [*laughter*].

Colman. But the irony was, of course, that the foetal fibroblasts were by far the most practically interesting cells that were being used in that whole programme, because there were huge numbers of them and they didn't have quite the problems of the embryonic cells in terms of longevity and robustness.

McWhir. You might have had cloning issues though … They might not have behaved so amiably if you tried to single-cell clone them.

Colman. Oh, but we went on to use them exclusively to do the genetic manipulation experiments that concluded in Diana [a sheep]. So, they worked very well.

Wilmut. Can I give you what you would recognise as the developmental biologist's thinking, as the way in which Keith and I viewed the Dolly experiment? I mean, having got the offspring from the embryonic cells in the procedure that we were beginning to have some confidence in [Megan and Morag], clearly our next objective had to be to use foetal cells and adult cells. The problem was, we knew how many we had to make, had to reconstruct. That was 200 embryos in order to have a chance of having a realistic assessment of the technology, and if

we wanted to compare two, we each had to have 200, so we simply couldn't afford enough sheep to do the two treatments in one year. I don't know whether we would have been able to squeeze the work in – possibly. What we set out to do was for us to do the foetal cells, as has been mentioned several times, and be prepared and able to take the additional embryo-derived cells and test. I guess it must have been about half way through the season, when we would've seen the foetuses by ultrasound from the foetal cell line, and perhaps, you know, one good line. We were learning from you [Alan Colman] that you'd only got one line to test and therefore you had sheep to spare, so that's the context in which it became possible to do the adult line in the same season.

Schnieke. No, we had done the number of embryo transfers for those embryonic lines, as had been planned, and instead of another embryonic line, which we didn't have because of the contamination problem, we then used the adult cells. So the fixed sets of experiments, which we were supposed to do, were done.

Griffin. I remember an institute meeting where Ian put a proposal, to squeeze in some extra sheep in that sheep season, and I think the cost was about £15,000, you must have been there, at that same meeting.

Archibald. I hadn't reached the elevated heights of the management by that point.

Griffin. I'd contradict it [*laughter*].

Wilmut. So in that one season – I'm looking at the source of knowledge – in that Dolly season, were there lambs born from both embryo-derived …

Schnieke. All of them, all three! [foetal fibroblasts, embryo-derived and adult mammary cells].

Ritchie. There were actually seven sheep born in that year.

Lovell-Badge. So which sheep were those?

Ritchie. The three cell lines that were successful were embryonic, foetal and adult, so we got four from the embryonic, two from the foetal and one from the adult line.

Lovell-Badge. And two of those were Morag and Megan?

Schnieke. No, the embryonic cells and the adult cells came from PPL, the foetal fibroblasts from Roslin, PPL paid for the experiments with the embryonic cells and the adult ones. For the foetal fibroblasts, Roslin did.

Lovell-Badge. But the cell lines hadn't been manipulated in those other sheep?

Colman. No.

Lovell-Badge. So they were, sort of, the proof of principle.

Schnieke. That was the next thing to do.

Lovell-Badge. There are lots of figures we know about Dolly so there's no point in reproducing those. But obviously Dolly generated a huge amount of publicity, are you saying that came as somewhat of a surprise, to some of you?

Griffin. No, I think you have to be in the centre of a media storm to know how intense it is, and Dolly was the tenth biggest news story in the world that year. Knowledge within the institute was kept to a very small number of people: right up to maybe two or three weeks before, I didn't know anything about it.

Lovell-Badge. Before the publication?

Griffin. Before the publication. I don't think it was ever discussed at any of the Institute committee meetings. Because of the experience of Megan and Morag, we started discussions with PPL and their PR company, De Facto. Ron [James, CEO of PPL] was the principal spokesperson for PPL and I think Alan [Colman] was in reserve. I was hoping to avoid any contact with the press and just manoeuvre in the background. We had media training for the principals including Ian, and De Facto got themselves all organised, and the whole thing was being planned to coincide with publication on the Thursday. The preceding Friday, the

press release was put out, embargoed, and that prompted pressure from New Scientist to send a photographer to take a picture, because they didn't want to be a week and a day behind the pace [being a weekly publication]. We were all geared up to start handling press on the Thursday, and Ian, you got a phone call on the preceding Saturday.

Wilmut. Almost midnight. Trying to think who phoned …

Griffin. Well, I remember I was watching a rugby league match on TV at about four o'clock in the afternoon and Ian phoned and said The Observer was going to break the story the next day. So on the Sunday, I went into Roslin to try to handle the inevitable press interest, half past nine on Sunday morning, after we had seen Dolly on the front page of *The Observer*. Ron James was at the south coast, he was diverted to Basingstoke where De Facto were based, and we just handled phone calls from then, for weeks and weeks and weeks. It was the most intense period. The staff didn't know anything about it, until they saw it in *The Observer*, and some of them were pissed off with that.

Lovell-Badge. None of the staff knew about Dolly either at that point?

Sang. The majority didn't.

Lovell-Badge. I'm going to tell you something in a minute.

Archibald. I didn't come onto the executive until 2000, but I knew, I think … Maybe I knew from my trade union, saying you've got to be ready for this.

Griffin. I wasn't aware of that, but by nine o'clock on Monday morning the car park was full of satellite vans, NBC, CBS, ABC, BBC, ITV, everybody and their aunt. It was an incredibly intense period.

Wilmut. We had a staff meeting on Sunday morning, didn't we?

Griffin. Yes, there was a staff meeting at quarter past nine, which some of you would've attended. I didn't attend it since both Alan [Colman] and I got dragged into dealing with the media. We kept the number of people that were dealing with the press as small as possible so we could maintain a consistency of message, which was absolutely essential, I think we could've been badly mauled if we hadn't handled it as well as we did … There's one particular instance, my secretary Francis Frame was on the phone, and every time she put the phone down, it would ring again, and that was probably true initially of every secretary at the Institute. One time, I overheard her saying 'Ah, sorry, Ian Wilmut can't handle it, he's got too many things, just give me your name. Harold Shapiro?' He had just been appointed Bill Clinton's Commissioner for Bioethics or whatever, and Bill Clinton had asked him to report on the ethical implications of Dolly. So I said 'No, no, don't put the phone down on him!' [*laughter*] We set up a meeting or at least a discussion over the phone with Ian but, yeah, it was a mad, mad time.

Colman. I think it had leaked within scientific circles. The publication was supposed to be February 27th, 1997, and I was at a meeting in Florida in January, and in the swimming pool someone swum up to me and said 'We know what you've been up to' [*laughter*].

Lovell-Badge. Well, I actually knew. I guess it was the autumn of 1996, because I was involved in running a course on embryology and transgenics in Hong Kong, and Keith Campbell had come and given a talk about it. He was supposed to be giving a talk about Megan and Morag, but at the end of the talk he told us all to keep it secret [*laughter*]. And it did remain secret.

Sang. Yes it did, I think most people in the institute didn't know. There were some people hinting that there was something that we didn't know, but we didn't know [*laughter*].

Schnieke. That's really surprising, because everybody at PPL was so excited when it happened.

Lovell-Badge. Alright, I guess we've gone off a little bit ahead of ourselves in

some respects, but, in terms of the other sheep that followed closely …

Wilmut. Holly would be next, wouldn't she?

Lovell-Badge. That's right, Holly and Olly

Schnieke. Yeah, the next step really was to prove that you can genetically modify them, and I remember going to Ian and asking if they were planning what to do next, otherwise we would like to do another experiment. I think there were still problems with the contract. We discussed if we don't start the experiments now, trying to make the transgenics, we come into the season problem again, and you [Ian Wilmut] just finally said, okay, let's do another round. So we prepared constructs for Factor IX in this case, made the transgenic lines, and then, at the right time in the [breeding] season, gave them to Ian, and it worked.

Lovell-Badge. So were those Holly and Olly?

Schnieke. Yeah, they were the Holly, Olly, Polly, Molly, they were Factor IX transgenic [sheep].

Lovell-Badge. Okay, they were all Factor IX, I thought Holly and Olly were not too important. Why did Molly and Polly become more famous that Holly and Olly, then?

Ritchie. [*laughter*] I think people were tired by the time …

Schnieke. It could have been because they were only neomycine positive which had happened because you select the cells, and it was a cotransfection, and so some of them were with cotransfected Factor IX and with neo [neomycin resistance gene] and there might have been some which had only neo.

Lovell-Badge. I thought that was Holly and Olly that just had the neo and Molly and Polly, but …

Schnieke. And that was really to prove that you can use the technology for making transgenics. They were all made from foetal fibroblasts.

Colman. Perhaps I can ask about the naming of Dolly, because it really came from your staff, didn't it [*laughter*]? [*To John Bracken:*] Was it you?

Sang. We are recording this fact!

Bracken. Yes, but it was just an off the cuff the remark, to my colleague who was there when Dolly was born, and I just mentioned to him that we should call her Dolly after Dolly Parton, because of the connection with the mammary cells, and I never thought that it would ever be repeated. I certainly didn't repeat that to Ian, so it must have been Douglas [McGavin, at Dryden farm] because immediately you [Ian Wilmut] mentioned it to people in the press, then it became a far bigger story.

Wilmut. It was good.

García-Sancho. To follow up with John Bracken, I'm quite interested in the process of looking after these animals and approaching them from a veterinary science perspective. Because we've heard a lot about the science, the laboratory science, so how was the experience of looking after the cloned sheep?

Bracken. The only animal that was different to every other animal was Dolly, and that's simply because of the media attention she got, and when the media came they wanted to photograph her, and the best way of getting her into a position was to give her food, so she associated the media with food, and that is how she became more … Well, she didn't become a pet but she'd certainly become far more friendly to people and would approach the gate and stand up into her trough. If you went into the place with all the other sheep, she would be the one that would be standing up looking, but it was association with food.

Ritchie. John, I think that the first time that she was actually introduced to lots of the press, there is a very interesting photograph, where she is actually doing that, and I think she was a bit of a diva. But she'd never seen the press, she'd never seen lots of people before, and I think it just so happened that she was an animal

which was, if you like, very forward and did stand up in her trough and look for something to eat.

Griffin. The photo you're talking about was taken by a now award-winning photographer, Murdo MacLeod (*Fig. 8*), and I was told that his wife had to step down from her job to meet the demand for that particular photograph [*laughter*].

Schnieke. I think what might also be quite interesting is the critique about the Dolly paper, and how many people did not believe that this was a real experiment. I think I went to Alan [Archibald] and he explained to me how to do a microsatellite analysis, and so we had done microsatellite analysis for almost all the animals to show that they could've only come from the [cell] clones. We used maybe five markers.

Archibald. It wasn't very many, certainly

Schnieke. It wasn't very many, but [enough] just to have some proof, and afterwards we actually had to do an almost forensic analysis, to get the proof that Dolly came from the mammary cells and [it was] not a cheat, because *Nature* had put the wrong leg on the paper (see *Fig. 9*) [*laughter*].

Griffin. That was quite an exercise, and if we go back to the comment earlier about the cells coming from the Hannah Institute. Because we were able to get those cells directly from the Hannah, transfer them to …

Colman. Alec Jeffreys.[27]

Griffin. Yes, and it was also fortunate that that short paper came out in the same edition of *Nature* as the one describing the cloning of 50 mice.[28] The two things together stopped the criticism dead.

Colman. I think you expanded the number of microsatellite probes you used; you did nine or so finally, it was five to begin with, but the *DNA fingerprinting* was done completely independently by Alec Jeffreys at the University of Leicester.

DNA fingerprinting is a technique that is used to identify individuals by a profile of DNA characteristics. A small set of DNA variations that is tested for is extremely likely to be different in all unrelated individuals.

Archibald. I think when you guys cloned the pigs later on we did more then. What's interesting is, if you did it today, you would sequence everything, and I suspect there would be a little bit in the corner that would be different, a very small bit. So it's possibly just as well we didn't have quite the penetrating tools we have now, back then. In fact just on, is there any material from any of them left, stuck in a freezer somewhere, a cell line, a bit of Dolly?

Griffin. Probably a piece of wool.

Colman. Maybe in Nottingham, because Keith, as you know, made more Dollies afterwards.[29]

Whitelaw. That piece of work is being written up for publication at the minute, the work that Keith went on to do, and there was precious little of anything left from the original.

Lovell-Badge. From the original cell line?

Whitelaw. From the animals, unfortunately.

Lovell-Badge. So Dolly is also in the museum right?

Sang. The same museum [National Museum of Scotland].

Colman. Oh yes, Tracy went down to London [to the Science Museum].

Bracken. When you're talking about trying to verify that Dolly was exactly what she claimed to be, we got an independent person to come and take blood from her, the head of anaesthesiology at the Dick Vet [The Royal Dick Veterinary School, University of Edinburgh]. He packed it, he sent it away, we, as employees of Roslin, never had anything to do with it, and it was obviously independently verified, with many strict guidelines to ensure that it was what it was.

Colman. When you were asked a question, John [Bracken], about the events after she was born, the husbandry, I recall that it was amazing that this one pregnancy held through to term.

Bracken. Right through the pregnancy.

FIGURE 8. Murdo McLeod's photo of Dolly's meeting with the press encapsulates the media frenzy her cloning caused once it became public knowledge.
© Murdo McLeod.

Colman. And so, we got worried that maybe she'd die on birth and things like that. I recall we had a 24 hour person who was just there with your guys, but after the birth I think Dolly wasn't well – she ran a fever after birth, and so you had your vets, and I think we sent our vet in just to add to the number, because we were so concerned that she might die in that crucial period just after birth.

Bracken. But certainly my recollection of her immediately after birth was that she was one of the most normal lambs that, you know, in my experience, so there was no concern on my part that she wasn't an absolutely normal lamb. I think you're talking of this fever that happened about day three or four, and after that, certainly in the first year, eighteen months, there were no other health issues whatsoever. So my recollection is that apart from this fever, which was very short-lived and only was a 24 hour thing, the rest of her youth, through to young adult was very normal, with no concerns for her health.

Ritchie. I think there was quite a number of us actually at the Dryden [farm] that day, because there were caesareans going on, so I actually saw Dolly and saw it get up on its feet and actually start suckling very, very quickly, just normal, just what I would've expected to see in any animal.

Bracken. And no issues as far as birth weight or anything, so she was normal in the real sense of the word.

Ritchie. Of course we were pretty certain that it wasn't a normal Blackface lamb [breed of Dolly's foster mother]. The colour certainly indicated that.

Schnieke. I didn't think you had that breed on the farm.

Ritchie. We didn't, it was very obvious that [Dolly] was a Finn Dorset lamb so we had no qualms about what was actually running around.

Lovell-Badge. So you're saying that others at the Institute didn't know that she

was cloned. Would they not have been surprised that you had a Finn Dorset lamb running in the field?

Ritchie. I don't think it … Come on, most people wouldn't have recognised it as a Finn Dorset anyway [*laughter*]. To most people, a lamb is a lamb and it doesn't really matter what it is. If you've got a decent shepherd, decent shepherds will recognise individual animals but most people say 'why did you use a sheep, we can't tell which one the cloned one is?'

Schnieke. All three were from different breeds. All the mother animals we used were all Blackface and then we had the Poll Dorset [Polly and Molly], the Finn Dorset [Dolly] and the Welsh Mountain [Megan and Morag].

Griffin. It was a good choice of breed, very photogenic. If it had been a Texel, I don't think Dolly would've been [*laughter*] … quite as popular.

Colman. But you mentioned the business of the front cover of *Nature* with the wrong leg [one of the back legs of Dolly on the *Nature* cover was actually the back leg of her surrogate mother – see *Fig. 9*]. That was due to erroneous use of Photoshop, and of course, I don't think anyone associated with the paper got to see front covers so they couldn't correct that. But it's interesting to speculate, in view of some of the revelations that happened in all the years following Dolly about image manipulation, that there was a mistake in the Dolly paper itself. Two panels were identical when they shouldn't have been (in one of the figures of the cells), and it was corrected in erratum but nowadays people jump on that immediately and say 'oh, can't trust these people.'

FIGURE 9. Cover of the *Nature* issue where Dolly was published. The odd-looking hind left leg was from the surrogate mother in the original photograph. © Nature Publishing Group.

Wilmut. They've been redoing the display in the museum, so Dolly's not been on view for a while, and the museum has got a sort of depot down in Granton Docks, aircraft hangar size, so I went down there and was shocked to discover she actually had arthritis all along her spine. Were you [Bill Ritchie] aware of that?

Ritchie. Only when Andrew Kitchener [curator at the National Museum of Scotland] mentioned it. I know Andrew very well.

Wilmut. Because there was a full histopathology done on her after. It's really obvious, but we certainly didn't detect it when she was alive, I don't think.

Ritchie. Yeah, but it's one of these things that seem to be fairly common in animals which are housed and which aren't getting a lot of exercise. I think Andrew Kitchener has done quite a bit of work on that.

Lovell-Badge. Was it in all four legs or just the hind legs?

Wilmut. I didn't go into that detail, the spine was what shocked me most.

Ritchie. I think mainly the hind legs, but it clearly was in almost all of the joints.

Whitelaw. Just to echo Bill [Ritchie], Andrew's got a theory that this [arthritis] is because the animal doesn't live an active life, and he's basing some of that on animals kept in captivity, in zoos, where some zoos have a very active regime for the animal, others don't, and there's an interesting story there. So I personally don't think that's a 'Dollyism', it's the fact that she was penned up and limited in her activity through her life.

Lovell-Badge. But the infection that killed her was …

Wilmut. Nothing to do with cloning.

Whitelaw. Jaagsiekte.[30]

Bracken. Something about the media. I think it was a very good move on Roslin's part, that they were so open about Dolly. I don't know how you restricted or allowed certain groups to come and see her, but we had lots of visitors and I think that openness and for people to be able to come and see her was a good way of ensuring the story of Dolly herself was a positive one rather than that she's under sort of lock and key and nobody's allowed to see her. I think that openness certainly helped the whole thing.

Lovell-Badge. But do you think if she'd been shy and retiring or grumpy and aggressive, the story would've been different?

Sang. Like the rest of the sheep [*laughter*]? Although this is the future, which we'll talk about later, I think the standard of openness in discussing what the experiments involved and what the outcomes were has really set the standard for Roslin that we've kept to, and that's been a very good precedent to have set.

Bracken. Because it would've been so easy for the bad publicity, which there was some of, to dominate the whole event and that certainly didn't happen.

Sang. But it gave us a lesson that if you're open and discuss everything, you get a much more positive response.

Griffin. It did for the Institute. It opened up tremendous opportunities. For example, both Ian and I were invited to discuss embryonic stem cell research by people who were clearly not in favour of any work on human embryos at all. A good part of the time after Dolly was born we were the loudest voice – if not the only voice sometimes – speaking up for human embryonic stem cell research, even though it wasn't core to the Dolly story. And the changes in law that allowed research into human embryos, I think we did contribute quite a bit to that.

Lovell-Badge. I think we're going to get onto that a bit later.

Ritchie. You're talking about the openness, and I'm sure Harry had told me a story about when Morag and Megan were actually born and the paper was published, that someone had come from STV [Scottish Television], and we'd been busy at that time and hadn't been able to accommodate them and tell them what was going on. And they said 'Well, we'll make up the story', and they did. I think you'd been speaking to the BBC as well, and by the time the story had reached the ten o'clock news, the BBC had the story, STV couldn't then say that this was all secret work. But I can remember actually seeing it, and seeing pictures of security cameras and saying that this work was secret work at the Roslin Institute. That may be what's helped in the subsequent Dolly furore, perhaps to be a bit more open.

Whitelaw. We did miss an opportunity, when we were phoned up and asked 'Can we speak to Ian Wilmut?' we should've answered 'Which one?' [*laughter*].

Colman. But the press was very scurrilous, sometimes very amusing but sometimes rather mischievous; for example when there was this issue of mitochondrial DNA, and the fact was that Dolly was made with a Scottish Blackface egg, and so you would expect to find some Blackface mitochondrial DNA in her, and indeed it was found. I think it was Steve Connor of *The Daily Telegraph* [it was *The Independent*] who penned an article with the headline 'Dolly is a fake or a fraud.'[31] Well, that's not helpful. On the other hand there was the bit when Alec Jeffreys published the DNA fingerprinting that tells you there's a 1 in 10^{10} chance that this DNA doesn't come from this individual but another one. I did some calculations that showed that there couldn't be more than 106 Finn Dorsets in the world, and foolishly told this to a reporter. Next thing I see is the headline 'Dolly's mother might have been an extra-terrestrial sheep!' [*laughter*] – now that's funny!

Lovell-Badge. Alright, shall we do Cupid and Diana?

Schnieke. Yeah, that was sort of the logical follow up, we had made the transgenics, and then we had to prove that we could do gene targeting. We had tried,

at the same time when we made the transgenics, to target *HPRT*, but as livestock seems to be able to use a different pathway, this wasn't easy. We did that with [David] Melton[32] and he was doing it in parallel in mouse (where it works), and after we tried it in sheep, there were also publications on some other species, which announced it was difficult. And then we had to decide on a different target. We were thinking that targeting in somatic cells would not be that easy, so we wanted to have some target gene which is highly expressed. We thought about actin, and then went for collagen, which of course is very highly expressed in fibroblasts. We then did targeting downstream of the collagen, first just the marker gene, and then also placing one of our transgenes into the same locus, and then the next season generated cloned sheep from these cells.

Hypoxanthine-guanine phosphoribosyltransferase (HPRT or HGPRT) is an enzyme involved in purine metabolism. Defficiencies in the enzyme can cause severe hereditary conditions, such as the Lesch-Nyhan syndrome.

Colman. The reason we chose a locus downstream of collagen was because there were known human diseases where collagen was mutated, and we didn't want the first knockout large animal to be a diseased one, so rather than the actual gene itself we wanted to take advantage of the locus [the advantage being high expression of the collagen gene].

Schnieke. And I think collagen is now used quite often, to place genes there which should be expressed.

Lovell-Badge. And why the names Cupid and Diana?

Colman. Oh, God knows why [*laughter*].

Schnieke. So first, they were both males, so that was wrong [*laughter*]. Then somebody had the idea that targeting a specific gene had something to do with the goddess of hunting, capturing, but that wasn't from me, I think that was Ron [James].

Colman. I don't know. Could I just end this with just mentioning that names can be wonderful. The choice of the name Dolly was a wonderful conception, and we continued the names and when we'd made the first five pigs, the cloned pigs, we named them. Dotcom was one of them, because anything with dot com on the end seemed to be doing extremely well, and the day that paper came out the market just fell completely [*laughter*]. There was a Millennium pig, Millie, there was Alexis and Carrel, and the origin of the choice of the names was a 1912 Nobel Prize winner [Alexis Carrel], who got his prize for the very delicate work on the human microvasculature, which allowed actual organ transplantation subsequently to take place.[33] He was a Frenchman who did his pivotal work in America. We learnt later on that this guy had been very big in the eugenics movement and all the boulevards in France named after him were currently being renamed. I got a letter from a famous French embryologist asking us to rename these two animals in view of this problem with Alexis Carrel, and he suggested Marie and Curie for the names. I just responded, I think I was just shooting from the hip and said there's only so much I can do for the French, and just left it as it was.

3. Funding Dolly

Lovell-Badge. We'd already begun discussing the relationships between Roslin and PPL before and during the cloning of Dolly. We also need to discuss the wider political and financial context in which the work was done: there were different government departments and funding agencies, MAFF,[34] BBSRC,[35] PPL got involved at some point. The Roslin Institute had a mixed funding stream, certainly then and probably still does, and I remember from my time on the governing council that questions were made about the different funding streams. Was that disruptive or helpful on the whole? Was it difficult to get funding for the Dolly programme?

Archibald. I think it was actually a very interesting time, thinking back, what was happening twenty years ago. Dolly was born in 1996, and the paper pitched

up in '97, and that was at the end of 18 years of attritional science funding under Thatcher and then Major governments. The Roslin Institute had been suffering cuts along with the other Agricultural and Food Research Council institutes over that long period. So 1997, I'd argue, was a sort of inflection point, the Blair government who put significant new money into research, and we've now reached the point where we've endured five years of flat cash, in terms of the science budget, 2010 to 2015. As from 1 April this year, BBSRC was the only research council to take a funding cut, and that's not in real terms, that's cash cut, so it's even greater. We're possibly heading back towards territory where government doesn't appreciate the value of research, it's almost like we've come full circle. Ian will remember well the issues after the Dolly project, when the paper came out and the subsequent funding challenge.

Wilmut. The funding for this project was cobbled together and I think I mentioned earlier on that it was a DTI [Department of Trade and Industry] precompetitive package that collapsed because one of the parts of the package was Martin Evans.[36] His lab was supposed to be trying to produce stem cells from cattle embryos, and we were supposed to be taking those cells and trying to make *chimaeras* with them by injecting into blastocysts. When we got foetuses, when the result came back, we obviously sent some of the cells there: the cell line which we'd been putting into cow embryos was actually probably rat … So it wasn't bovine and that package stopped.

Here, a *chimaera* refers to an animal made experimentally from two or more embryos or embryonic cells. Chimaeras can be made by injecing genetically modified embryonic stem cells into an embryo at the blastocyst stage, and if these cells contribute to the germline, some of the offspring will carry the genetic modification.

Lovell-Badge. So those cells had come from them because they were trying to do rat stuff at the same time.

Wilmut. Well, they would possibly have been used as feeders. They certainly weren't bovine, maybe I'm getting confused about what they were.

McWhir. In my memory they were mouse. I had exactly the same problem when I was working in Martin's lab, I was trying to isolate porcine and cattle ES [embryonic stem] cells, and I thought I had a pig line, but fortunately we karyotyped it before we told anybody. It turned out that it was mouse, so it's very easy, when you've got both cells and you're working in the same tissue culture, to get cross-contamination.

Wilmut. So we then got money from a European grant, 1994 to '96 or that time period. I'm sure the Institute put its hand in its pocket and gave us some money at some point, yes?

Griffin. Yes. As I said earlier, I think it was for the sheep, in relation to the sheep season.

Wilmut. We did get some MAFF money, but as Alan [Archibald] mentioned, on the day when we announced the birth of Dolly, MAFF said they were going to stop the funding, which seemed something of a PR own goal.

Griffin. There's a broader funding issue, or a tension that the Institute's always been under, ever since I joined the Poultry Research Centre in 1978. This comes out very strongly with every visiting group. We're supposed to be doing basic science, say the academic members, or we're supposed to be doing something useful for the industry, and all the work we've been discussing actually falls between the two stalls. It's a bit ironic that Dolly's creation is the single most important piece of basic science this Institute's ever delivered, in terms of its impact on the understanding of cellular differentiation. Alan [Archibald] has talked about the funding for the science project, but all throughout the period from 1980 onwards, funding from the Ministry of Agriculture, DEFRA, whatever form it took, has always been decreasing, and they've been moving away from providing funds for more basic science. Whether this project as a whole would have ever qualified for Department of Education and Science funding … I'm not sure it would've done.

Archibald. I'm sure the reviewers of such a grant proposal to BBSRC would've

FIGURE 10. The PPL building in Roslin.
© PPL Therapeutics.

said 'do it in mice first.' But I think one of the really important things, as far as Roslin was concerned, was that ultimately, when we sold the technology on to Geron, that funding package [that Roslin got from Geron in exchange of the technology] saved the Institute because that was the point when the DEFRA money completely fell off the cliff and essentially went to zero. That Geron money allowed us to survive that period until we picked up our position, getting good grants out of BBSRC. Without that I think the Institute wouldn't be here.

Lovell-Badge. How was the relationship with PPL at this time?

Colman. After Dolly we hired Keith [Campbell], he went to the dark side. Keith was unique in many ways and he of course made the pig project go successfully, but he always had itchy feet, and I think academia was more aligned with his personality in many ways. In 2000 he went to Nottingham from us, so he was with us for about two years and spent quite a lot of time in the US where the pig work was done. Dolly was the only time the share price [of PPL] ever got above the flotation, and it was about four days, I think it got over £5, but it was so close to the flotation that none of the directors, like myself, who had shares could ever make any money – some of the staff did, because they could sell their shares, which had been issued at low prices. But Dolly was the height, the zenith, I'd say, of the share price and it was downhill from there on, and we raised more money after that time, and we turned towards embryonic stem cell work on the side, while trying to move the products to the clinic. The manufacture of the biopharmaceuticals was very successful in a sense that we could make a competent preparation of alpha-1-antitrypsin at $16 a gram. For something that could go down into a person this was enormously cheap, so the concept was right, but the fact is that alpha-1-antitrypsin failed probably because the post-translational modifications were

not the same, the different sugars. What happened was the lifetime in the human blood, the turnover was much greater with sheep-made human products than the human-made products, so we went to a different indication which was CF [cystic fibrosis], but it didn't work there, didn't do what it needed to do. It was always an untested hypothesis and it didn't work so, eventually, I left in 2001. When did you leave Angelika [Schnieke]?

Schnieke. Very early in 2003.

Colman. In 2003 [PPL] was acquired by hedge funds and that was the death knell. I mean, they acquired it because they wanted to …

We're supposed to be doing basic science, say the academic members, or we're supposed to be doing something useful for the industry, and all the work we've been discussing actually falls between the two stalls.

IAN WILMUT

Schnieke. Get the patents.

Colman. Yeah, get the patents and split the company, so the American side survived that, and got taken over by others.

Lovell-Badge. The patents were bought in 2004 by Pharming, the Netherlands company. But then something ended up in the University of Pittsburgh?

Griffin. Has anybody taken the technology and produced a product that's gone on to clinical trials?

Colman. No, not from PPL.

Griffin. Not anybody in the world?

Colman. There are pharming products in very small niche markets, but they have been approved and they are used, yeah.

Lovell-Badge. And is that because they are difficult, because people don't like them? What's the reason?

Colman. The time to market is much extended when you turn to large animals – I mean, cloning doesn't affect the gestation time of a sheep or a cow, it's still a long time. Cell culture methods improved markedly in the yield themselves, [became] much more controllable. A lot more competence in engineering has been devoted to getting cell culture products to the market, and so, really, the pharming concept was best with proteins that you needed in very large amounts, cheaply. Alpha-1-antitrypsin is a very good example. I don't think the coating factors were a good example because you didn't need huge amounts of those, and I don't think Factor IX ever worked, it's down to *carboxylation*.

Carboxylation is a type of post-translational modification of a protein. It primarily occurs in the proteins involved in blood clotting, such as *Factors VII* and *XI*, and *protein C*.

Schnieke. No, they all worked but you never got very high expression levels because you needed carboxylation. We did some *protein C* work, some *Factor VII* work.

Colman. So, they all worked well?

Schnieke. They all worked, yeah.

Colman. The niche we were aiming at was, originally, this large volume, low cost, and just took too long. And the one [protein] we developed didn't work, unfortunately, but we didn't know that to begin with.

Archibald. Well, there was also the suspicion that when PPL had the relationship with Bayer, Bayer already had alpha-1-antitrypsin as a product they were trying to develop with different technology and that didn't seem like a great idea.

Schnieke. Yeah, that's true.

Archibald. And then, as you say, the hedge fund: basically the money [PPL] had in the bank was greater than the value of the company, if I remember correctly.

Colman. Yes, yes. But it did well for a time, and when it floated in 1996, people said you could float £10 notes at that time, so capitalism helped us to begin with and took us down in the end, and you just had to accept that.

Archibald. Am I correct in thinking that when the company was founded, the

Scottish Development Agency [SDA],[37] as it was then, were a key broker in getting the company started? And that there wasn't really that kind of government level intervention in other parts of the country, so it was a kind of advantage of being here?

Colman. I think it was. I was already here with Helen [Sang] and learnt about John Clark's work, and the SDA sure did help, but it wasn't as if there was any thought of having that work done anywhere else in the country, because the inventors were at Roslin. When we started Caledonian Transgenics, we started down in Birmingham, where I was at the time and some of the people were. Then, with the large animal work, Andy Carver had to drive up in these cars to perform his microinjection which he learnt from you guys. The molecular biology was being done in Birmingham and ultimately it just didn't seem sensible to have work going on two sites. Then we moved the company up to King's Buildings [in Edinburgh]. That was where we first started. Subsequently we acquired a purpose-built building on the Roslin site, surrounded by non-transgenic sheep, which we joked acted as decoys for any persons who would wish us ill. People thought, 'Ah, do you keep your transgenics near?' and we said, all the sheep in the fields round Roslin are decoys. Really, it was very enjoyable, working with the Roslin Institute, all of us on the same site.

Wilmut. Paul Simons was the person who started the gene injection work in mammals at Roslin.

Colman. Where, in your place?

Wilmut. Yeah, I mean, when we started, the work you mentioned from Brinster and colleagues at the USDA [labs in Beltsville, Maryland], was published, on actually how to do it, and we didn't know.[38] It was Paul [Simons], with a little bit of help from me, who found the pronuclei, and his PhD had been injecting into cells, so oocytes were a handy size thing to work with in comparison to cells. But of course they are lipid filled, so you can't easily see the pronuclei, and that was the knack, and he did all the early gene injections for us.

Lovell-Badge. Yes, I remember visiting once and he showed me how to centrifuge the eggs to separate out the pigment and lipid. So clearly there was this interaction between the different sides of science, the molecular biology, the cloning work, the reproductive biology, cell cycle stuff. I assume everyone thinks that was very important for the success, both of Roslin and, at that time, PPL, because you had good interactions.

Colman. Yes, there were tensions at times, inevitably, but it was very productive for us, and hopefully for them, too. But the breakthrough technology came from Roslin and we learnt it by copying them, and exploited it. It was a very nice time, I think, because everybody was relatively young then [*laughter*].

Lovell-Badge. And did that relationship maintain itself throughout?

Colman. Yeah, at the end, obviously, there was less interaction because …

Schnieke. Geron,[39] I think once Geron came.

Colman. Yeah, once Geron came we started doing the nuclear transfer out of our facility. It was a lot more expensive for us, because all our sheep were flown in from New Zealand on a jumbo jet.

Lovell-Badge. Was this to be scrapie-free?

Colman. Yeah.

Archibald. And one of the ironies of that, of you shipping in scrapie-free animals from New Zealand, was that your farm at Ormiston was within sight of the Institute's former farm at Skedsbush, which is where the Institute of Animal Health's scrapie-infected flock was going [*laughter*].

Colman. That was just one of the hazards. We were trying to make this clinically qualified protein and we were worried about coal dust getting into all the

equipment filters and everything. There were all sorts of challenges.

Wilmut. Robin, if you were to make a comparison and ask about relationships with PPL and with Geron … I'm biased, but I think the relationship with PPL Therapeutics was much more collaborative than with Geron [*general affirmative noises*].

Capitalism helped us to begin with and took us down in the end, and you just had to accept that.

ALAN COLMAN

Lovell-Badge. So they [Geron] were brought in because you needed money for …

Wilmut. No, they licensed our technology and the Institute got a large amount of money, I don't remember the numbers but it was a lot of money. The sale was assessed by some parliamentary committee, wasn't it?[40] And the Institute got a pat on the back for what it had done, that may be right, but part of the deal was that Geron would fund work, and the way in which it would be managed would be that they would agree a year or two's work, and they'd come and assess it every now and again. At the end of the first year they would make a long-term assessment and either indicate at that point that they were going to stop it in a year's time, or re-extend it back to two years, so that you never had less than a year's notice as to when things were going to be stopped. And they came one April and said it [the nuclear transfer work] was okay, came back in June and said they were going to stop it, and it was stopped in August.

Colman. Could it be that it wasn't worth pursuing at the Institute?

Wilmut. We felt that they'd bitten off more than they could chew, because they had the telomerase work, human embryo stem cells, which became their biggest thing for a while, and nuclear transfer. What they did was they shifted the money from nuclear transfer to stem cells and so for a while Roslin had the biggest group of people working with human ES cells in the United Kingdom. It probably did the Institute quite a lot of good. Quite a number of individuals benefited from that opportunity. It was the people who were involved in the cloning, which had produced this wonderful opportunity, who were chopped off.

Lovell-Badge. What year was it that Geron came in?

Schnieke. It was after Dolly and so the reproductive cloning was just starting [in animals].

Wilmut. It would be a few years after Dolly, though, because what they actually did was to buy the company we'd started. The Institute started [another] company, Roslin Biomed, to develop the cloning technology and to licence the technology to various people including PPL, and they [Geron] bought it.

Sang. And Roslin Biomed had 3i investment,[41] and once you have that investment, the investors are in control, and they wanted to make the sale to Geron, isn't that correct?

Wilmut. Yeah.

Griffin. The CEO of Roslin Biomed was Simon Best, and Simon had been previously working on bringing transgenic tomatoes into the UK. So he had a rather complicated history of pop music business and transgenic tomatoes [*laughter*].[42]

Sang. Didn't they start up a company out of their stem cell business?

Colman. Yes, it's called Asterias and they're running a clinical trial now, on age-related macular degeneration, in the US.

García-Sancho. I wanted to, ask about the Scottish Development Agency and the role of these kind of agencies in promoting what was Caledonian Transgenics. Was that a response to the scientific policies of the time on prioritising applied research and commercialisation of basic research?

Archibald. When Rick Lathe, who was the original driver of making proteins in milk, was running the molecular biology group, because Rick had come from a

biotech background we were very active trying to sell the idea to biotech companies and big pharmaceutical companies before we'd done any work at all. That included speaking to BTG who were supposed to act as a kind of UK-wide agency for developing new biotech.[43] My recollection is that the Scottish Development Agency – this is 25 years ago – had had successes in terms of creating Silicon Glen,[44] and they were looking for something in the biotech space, they wanted something to do with biotech as well, which is why we managed to attract their attention. They played some role in raising funds getting someone to say they were going to put some money in – makes it easier for the second person or the third person to put money in. I think it kind of felt like that, that they'd come with the catalyst to getting the funders on board, to create what, as Alan [Coman] said, was Caledonian Transgenics at the time.

Colman. It went down really well in the US [*laughter*]. Caledonian, they had no idea what that meant.

Archibald. We made up some tartan logo for it as well if I remember correctly.

Colman. I'm not sure about that.

Archibald. There was some notion, well, maybe it was a spoof tartan thing we did, I can't remember.

4. Species choice

García-Sancho. Alan Colman has mentioned that, after Dolly, PPL hired Keith Campbell to run a programme on pigs. I just wanted to ask whether in the early years of the pharming programme or even before, there had been work aiming to produce genetically-modified pigs. You had talked about sheep, about eggs and chickens, about goats, but what about pigs?

McWhir. Ian will correct me if I'm wrong, or Alan [Colman], but I think the follow on work in terms of species progression from the supermouse[45] in 1982 was pigs, with the same [genetic] construct, was it not?

Lovell-Badge. But that wasn't pharming.

McWhir. Sorry, did you specifically mean pharming?

García-Sancho. I did say pharming, but I meant more generally the roots of work on genetic modification of pigs.

Archibald. Well there was Bill Wilander who worked with the American Red Cross, and his pig project was to make, I think it was human *albumin* in pig blood. It was to produce product for enhanced plasma.

Human serum albumin is the most common protein in blood plasma.

Whitelaw. It was protein C.

Schnieke. But it's not so easy to milk a pig, so for pharming the pig is not the best model.

Archibald. Although someone did do it, someone did.

Colman. It's not easy, they're dangerous. [*laughter*]

Sang. There was somebody who wanted to make proteins in the semen of pigs, because you can get semen from transgenic pigs.

Colman. A little known fact.

Bracken. Speaking about the pig, versus the sheep, it is a completely different ballgame, actually, producing work for nuclear transfer when using pigs. The animal is bigger, it's more costly to keep, the superovulation is better but is more difficult to achieve, the anaesthesia is more difficult, the surgery is more difficult, and the fact that the pig itself, even if you get it pregnant, has a mechanism to stop a pregnancy if there's less or more than four or five viable embryos. All these things are against using the pig, compared to the sheep, unless you really want that as a species, and then you have to tackle all these individual problems.

García-Sancho. Were there conversations between the veterinarian people and the scientists, in order to choose one model organism? Would you share that in-

formation with the scientists doing the transgenic work?

Bracken. I think the pig was initially used because of the xenotransplantation, that was why they moved from sheep to pigs. But as far as obtaining the oocytes, we actually would superovulate the pig, bring it over to the surgery unit, do an initial scan of the ovary to check whether it had superovulated or not. If it hadn't superovulated, we would discard it from the group, but then after that, two of us would come in at midnight and scan these ovaries with the developing follicles, and we would scan them every two hours, initially, to find out when ovulation occurred because an ovary with follicles on it is very easy to see on an ultrasound scan, but when the ovulation occurs, because the oocyte has then dispelled the fluid, it's very difficult. So you could actually determine when ovulation occurred, so we would scan at midnight, two, four, and six, and we could then pick up a surgery list of when the animal ovulated, so the ones that ovulated first were the ones that we did surgery on first, and then at six o'clock, when surgery started if they hadn't ovulated these were then used as recipient pigs. But that took a lot of learning and effort to do, because we would be doing three sets of pigs in a week, so two of us would be coming in three consecutive nights to work through the night, then do the surgery and that in itself was a big, big effort to achieve what was far easier with a sheep.

Whitelaw. What was PPL's drive into pigs?

Colman. Xenotransplantation, the major thing was to knock out a gene called *alpha-1,3-galactosyltransferase*.[46]

Alpha-1,3-galactosyltransferase is an enzyme responsible for the synthesis of the sugar galactose. It is implicated in immune rejection in human-to-pig xenotransplantation.

Whitelaw. So would it be fair to say at that time that there were several players in the bioreactors [i.e. pharming]? There was yourselves, there was Pharming, there was [the company] GTC, and American Red Cross, and you each picked different species. Was there a driver for that or was it a business decision?

Colman. Well, we picked sheep because you [Roslin Institute] picked sheep *[laughter]*.

Whitelaw. Well, we had that expertise. Because American Red Cross did pigs, GTC did goats, and Pharming did the cattle, didn't they?

Colman. Yes.

Whitelaw. So was that wrapped up in the patent positions or was it just where you were?

Schnieke. No, I think the patents were more on the promoters than the species.

Colman. We were always battling with Pharming over intellectual property issues and we usually won, but they bought all our patents at the end when PPL went belly up, it's sort of a pyrrhic victory, of course. Their first published animal was Herman the bull, which was, unfortunately a male so it couldn't make milk in the conventional way. Well, we found ways of getting milk out of males, I have to say, which we didn't pioneer, but we could evaluate a line through the milk from the bull.

Whitelaw. So the drive into pigs was purely xeno[transplantation] then, around the world?

Colman. Absolutely, absolutely xeno.

Schnieke. You use the best model for the project you want to work on.

Colman. We had considered, because it had been mentioned in the literature, ostriches [*laughter*]. It was serious because physiologically, particularly the cardiovascular circuits were more human-like, because the ostrich walks on two legs.

Whitelaw. Can you catch them?

Colman. People farm them.

Wilmut. There weren't any volunteers.

Lovell-Badge. So, how many cells in a newly-laid ostrich? [*laughter*]

Sang. Actually probably about the same. I was talking to Megan Davey[47] yester-

day about comparing quail and chicken and actually at the very earliest stages of embryogenesis, in quail, chicken and emu, the embryos are the same size.

Colman. We never got that far in that evaluation of ostrich eggs.

Whitelaw. You couldn't catch them?

Colman. No, we never got serious about them.

Archibald. So in terms of pigs for nuclear transfer, the Roslin-PPL team were second, we were scooped?

Colman. Well, actually, that was quite interesting, yes and no. This work was led by Keith [Campbell]. The first five pigs, no doubt about it, were cloned by us, and they wrote up a paper by Dave Ayares and colleagues, who worked at the PPL facility in Roanoke, Virginia, USA. They wanted to submit the manuscript to *Science*. As CSO of the company, I vetoed that (big mistake) and said, why don't you submit to *Nature*, because *Nature*'s British and we had a tradition with *Nature* at the time. Also, *Nature* got in contact with me because they'd heard rumours about this work and they said, well, if you submit to *Nature*, we'll give you a 48 hour review, we'll give you a front cover if it's accepted and a News and Views opinion piece. So I said yes, and so we redrafted it, submitted it to *Nature* but they didn't do it in 48 hours, they did it in a much longer time, and then they sat on it, they accepted it but sat on it, and the reason they sat on it turned out to be they wanted to publish a paper by Robin Weiss on viruses in pigs, so they wanted to have an article disclosing the first cloning of the pigs to facilitate making the perfect pig for xenotransplants next to an article highlighting the dangers of putting pig organs into people. Meantime, a Japanese group, who started cloning their pigs after our pigs had been born, submitted a one-pig paper, if you like, to *Science*. *Science* accepted it in record time and got in touch with me, and asked if I could write an opinion piece on it quickly for them. They perhaps shouldn't have chosen me, because I immediately contacted *Nature* and said "Look, you buggers, this is what you've done to us by hanging on to our paper." So what *Nature* did immediately was to publish it online, so the electronic version came out on the same day or a day before, maybe, the paper version of the Japanese group, who had not made an online publication. So, yes and no, that's why I say yes, we were definitely first but we were delayed by *Nature*. They just held onto it so they could put the two papers together, and we weren't informed about that at all.[48]

Schnieke. Was that the knockout paper?

Colman. No that wasn't the knockout, that was the cloning paper. We weren't first on the pig knockout paper, we were first on the double knockout, second on publishing the [first knockout paper], to an American group. But PPL were the first group in the world to make a large animal knockout [a sheep].[49]

Lovell-Badge. So no one ever made embryonic stem cells from the pig, or sheep?

Colman. I don't believe so, I think iPS cells have superseded them, and they make those from those species.

Whitelaw. But there's only one group that's shown that such iPS cells will go into the germline. Steve Stice has showed that, there's a little bit of controversy over that, in that the paper for germline transmission [i.e. the foreign gene passing on to the offspring] was validated by *PCR* rather than a *Southern blot*, which I think the academic community would have bought, and certainly those cells are now no longer able to do so.

Polymerase chain reaction (PCR), is a method for amplifying DNA in a sample, which can also be used to identify presence of specific DNA. *Southern blotting*, invented by Edwin Southern in Edinburgh, is a method for identifying and visualising a specific DNA sequence in a sample.

Colman. So how did they do it, was it chimaera work with the cells, or what?

Whitelaw. Yeah, it was chimaera work and then germline transmission, if I remember correctly. So it's still a challenging, very much a challenge to get any robust cells. In fact, Jim [McWhir's] cells, the TNT cells, are as good as anything else that's ever come out, really, from the academic community.

McWhir. Oh no, I don't think so. We didn't have extensive differentiation detail.

FIGURE 11. Five piglets cloned by PPL in 2000: Christa, Carrell, Millie, Alexis, and Dotcom. Courtesy of Alan Colman.

Whitelaw. That's correct, from the point of view of differentiating those cells into lineages there's quite a lot of progress in the iPS but actually going in and making an animal, that's still not possible.

Lovell-Badge. Pharming in birds, is that happening now?

Sang. Yeah, we still have it ongoing, actually. The first therapeutic product made by extracting from the eggs of genetically modified hens was licensed in Europe and the US last year. It's for a lysosomal storage disease so it's sort of an orphan drug route, that's how it's gone through the regulators and it's being sold. The company that started out as AviGenics became Synageva, and was bought by Alexion which is quite a bigger company, last year, for a lot of money. So there's now a product from hens.

Colman. What is it, what part of the hen?

Sang. From the egg, the egg white.

Colman. The egg! So it actually works ...

Sang. It works, we did it several years ago and we can now make proteins in eggs. We can make a gram, about a gram per litre.

Lovell-Badge. So that came out of the work that was done.

Sang. I think we worked in parallel. It's one of those things, it was discussed about 30 years ago, there are very few proteins in eggs and it's relatively easy to purify proteins from egg white, because it's relatively simple to mix the proteins. So if you know anybody who wants to invest we have a product that we're developing [*laughs*]. It's very interesting to hear about alpha-1-antitrypsin, actually, *glycosylation* might be better in the hen's egg than milk.

Glycosylation is a type of post-translational modification of a protein.

5. Dolly's legacy

Lovell-Badge. We've covered several areas now. Going back to Dolly and cloning, things we had around the same time were the first embryonic stem cells, and so this led to the idea of therapeutic cloning to drive patient-specific cells. There were also fears of reproductive cloning, and Ian [Wilmut], you were prob-

ably asked to provide evidence to various committees about this, and you know that initially in the UK, the Human Fertilisation and Embryology Act didn't really permit derivation of human embryonic stem cells, so there was a big campaign to get that Act changed, which happened in 2001, and that also permitted therapeutic cloning, somatic cell nuclear transfer.[50] This was all triggered by Dolly, so Ian, you were very much involved, I suspect.

Wilmut. In primates, including humans, the Roslin procedure doesn't work, the primate oocytes are different. Last year a couple of labs showed that primate oocytes are much more sensitive to inadvertent activation, so you get a partial activation, which then messes up the oocyte and reduces its ability to respond properly when you really want it to activate.

García-Sancho. People normally know what happened with Dolly and the nuclear transfer technology, but I guess less people would be aware of what happened afterwards and where the technology went and where it was applied and what it has enabled, and what lines of research it opened.

Griffin. There are two elements to that. One is, who is cloning now and for what purpose, and is it bringing in any significant revenue? And the second is more fundamental: what the consequences are of understanding that cells are much more plastic than we ever thought they were? The first one should be easy round the table: Who knows who is cloning what, at the moment?

Whitelaw. I'd say there's a third legacy in that it had such an impact on society that it accelerated the public's desire to see this type of science, biology and genetics in general, in the media. Prior to Dolly, it was very hard for you to get a story into any newspaper, and since Dolly, newspapers and media are presenting our science all the time. I think there was a step change at that point, and it was down to Dolly. I don't know if people agree with that.

Archibald. I think that's hugely overstated Bruce, science had mattered before Dolly.

Ritchie. But I don't think it happened so much in public, in newspapers.

Archibald. Yes, it did.

Sang. There's also an element of coincidence as well as consequence, and nobody could pick that apart. But I do think it had a huge boost in a sudden freeing up of scientific, bioscience thought. You've broken down barriers here and we can think about doing things in a different way, and we have things like that happen every now and then. At the moment the artificial nuclease, the *CRISPR-Cas9*, is having that same sort of freeing up effect, of letting people think about all sort of different ways they can use the technology to make faster gains of knowledge.

CRISPR-Cas9 is a bacterial gene editing complex that has been coopted for genetic engineering since 2012. It enables hilghly precise genetic changes.

Colman. I think I take a slightly different view. For me, the principal legacy was the confidence it gave the scientific community that it was possible to reprogram an adult nucleus down to a pluripotent state, much more embryonic. I feel that the motivation that the creator of iPS cells, [Shinja] Yamanaka, had was in part inspired by Dolly, inspired by the frog work, inspired by embryonic stem cells being made from humans and things like that. All these things were important. You know, for 30–40 years before 2006, when Yamanaka's first reports came out, I had been under the impression you just couldn't separate the nucleus from the cell, get it reprogrammed from the adult to embryonic state. I thought, adult to the larval state, yes, but there was something special about the adult state, it just won't work, and that was broken by one experiment. It was very surprising, and that, to me, was the scientific legacy of that demonstration.

Whitelaw. So, although that's probably the biggest thing, there are companies which are still using cloning, but finding out the details of exactly what they're doing is extremely hard. There are research groups who are using cloning technology – Angelika [Schnieke] across the room is a wonderful example of that –

and there are still pockets of people trying to research nuclear transfer per se, although that's a very small part. So the actual technology is there and is used, Alan [Colman]'s correct. Everyone, I think, agrees that the big transformation is that it has made us believe that we can do things to cells that we couldn't do before, and that's opened up … Well, it's fuelled the human stem cell effort.

Lovell-Badge. Regenerative medicine in general?

Whitelaw. In general, absolutely.

Lovell-Badge. I'm thinking about the timeline, human ES cells were in 1996?

Colman. Primate ES cells were 1995, human ES cells were 1998.

Lovell-Badge. And then the two items together [Dolly and ES cells] with the idea of somatic cell nuclear transfer, maybe therapeutic cloning. There was almost an immediate response to try a worldwide ban on reproductive cloning, even though I still can't think of any reason for doing it.

Colman. Vanity.

Lovell-Badge. Vanity is the only one [*laughter*]. Then, I guess iPS cells weren't until 2000s.

Colman. 2006 was the mouse, 2007 was the human, but the lead up to that was quite interesting. Yamanaka spoke at conferences I went to, and we all knew what he was doing, and just no one thought it would work, the way he was doing it. But the fact he was doing it was because he felt something would work. I was just amazed it worked, it seemed such a crude way of conquering a problem that had been so refractory for so many years, but it worked.

Lovell-Badge. A lot of work has gone on looking at reprogramming to make iPS cells and the mechanisms involved. I guess people haven't done so much on somatic cell nuclear transfer, and looking at the mechanisms. Although, I guess, John Gurdon's still …

Colman. No, I don't think John is really …

Lovell-Badge. He's interested in how you reprogram a somatic nucleus back to an embryonic state, he's still using frog eggs.

Colman. He's still using frog eggs but I think he's addressing different questions, not trying to work out how the reprogramming takes place.

García-Sancho. Now that we are discussing the legacy of Dolly, it looks like the main knowledge that was gained [from her] was about the plasticity of cells, about the possibility of programming and reprogramming cells. Could one of the messages of the story be that even though in the 1980s and 90s there was a push for applied science and the creation of spin-off companies, scientists continued doing basic science: their main motivation was looking at the fundamental mechanisms of the cell rather than producing animals that may have a commercial output?

Sang. I think you're right, in the past and probably in the future the Roslin Institute will be pressured to become more applied, but I don't think you have a viable research institute if you don't have your feet in fundamental research, so we've always managed by nifty footwork to keep some basic research going as well as the applied research.

Schnieke. I think I would also say that after Dolly almost all knockout, transgenic, any type of genetically-modified animal in livestock was done with the nuclear transfer. People have given up on microinjection because it was so inefficient. They [still] do the viruses in the chicken, that's a separate one, but almost everything else is made with nuclear transfer. There are still people working on xenotransplantation, there are more and more people working on porcine models for human diseases, and that's in the area of diabetes, of cancer, of cardiovascular disease, of muscular dystrophy, cystic fibrosis, and it's all done with nuclear transfer. Right now, the CRISPR technology is coming in and it's a bit of a mix,

depending on what you want to do, you either do it in combination with nuclear transfer or you do it directly in the embryo.

Colman. Do you think it'll be completely superseded? That most of the uses that currently are nuclear transfer will be taken over by CRISPR?

Schnieke. Depends on your question. For example, for xenotransplantation you need to get a large number of transgenes into it, CRISPR doesn't really help you there. If you just want to knock out a gene or make a small modification, it is fine.

Whitelaw. I'd say that, from an agricultural endpoint, the CRISPR and direct injection have certain advantages. This doesn't exclude cloning, in the biomedical world there are many reasons why nuclear transfer will still be a dominant force.

Ritchie. One of the other fields where nuclear transfer will still be used in is endangered species. I saw a publication not long ago on several different endangered species, in which they were using cells from nuclear transfer and putting them into a rabbit. So, there's still some experimentation going on there.

Lovell-Badge. And also for valuable animals, whether it's cows or dogs.

Whitelaw. Yeah, possibly.

Lovell-Badge. I mean, South Korea …

Whitelaw. Yeah, in the pet world there are different drivers, aren't there? Alan [Colman]'s mentioned already that some companies are just run by rich individuals. And that certainly dominates the pet world.

Lovell-Badge. Not just pets but also police dogs and things like that, sniffer dogs. I thought that's what they were cloning.

Archibald. Come on!

Colman. Sniffer dogs?

Archibald. These are the ones that are finding the cheese sandwiches instead of the turkey sandwich [*laughter*].

Ritchie. That's very good PR, if you could clone something like that.

Wilmut. So what would the Home Office view be on nuclear transfer?

Lovell-Badge. You'd have to have a good reason for doing it, it depends what.

Wilmut. When I was last thinking about this and talking to inspectors, that was the answer – you couldn't just do it to multiply [organisms], and I don't think it's regarded as part of normal agricultural practice, is it? So you can't just do it in conventional animal breeding, can you?

Lovell-Badge. In the UK you can't.

Sang. And in the US?

Lovell-Badge. In the US you can.

Archibald. I think the way the law stands at present in Europe or the UK is you cannot put offspring of clones into the food chain never mind clones themselves.

Lovell-Badge. That's a European law, not a UK law.

Griffin. That would be clones by nuclear transfer, not by embryo splitting.

Archibald. They don't know what they're doing, basically.

Griffin. Ian [Wilmut], what's the current status of the health of clones?

Wilmut. I don't know. You probably know more than I do, Angelika [Schnieke].

Schnieke. It's still an unpredictable thing. Sometimes modified cells get almost the normal litter from the pigs, and all the animals are healthy, and with other animals, with other cell clones, we have problems. Then we get smaller litters or they have epigenetic modifications, and it's very difficult before we do the nuclear transfer to know what happens. It is getting better. It has become more efficient, we have 50% pregnancy rates, but we really can't completely predict if we'll get a healthy animal or not.

Griffin. And does that vary with the cell line?

Schnieke. It could also be with the clone. We don't have cell lines, it's all primary cells, and even if you have one clone or another from the same primary

cells, you could have a difference, but it can be different reprogramming, it can be anything. But it's efficient enough.

Lovell-Badge. How many attempts doing nuclear transfer and how many healthy animals born?

Whitelaw. How many reconstituted embryos for one live birth?

Schnieke. How many reconstituted embryos? We actually only have one nuclear transfer session a month and that's not every month, so it's not that often, and we get a reasonable number of lines.

Whitelaw. To summarise, the inefficiencies that were seen in the early days are still there. The vast majority of work has been done on the pig, and there have been improvements. Some of the commercial setups claim to have made significant improvements but some of the original issues are still there, at a variable rate.

Schnieke. Once you have the cell isolate which you know works well, then usually you get your animals, but it's never 100% guaranteed.

Whitelaw. And again it depends what you're trying to do. Take Angelika's work where she's trying to generate human disease models. You don't need a hundred founder animals, you need one.

Schnieke. Just one.

McWhir. I was interested in your comment that once you get a line which works well, it continues to work well. It does suggest that there is something about the donor cell.

Schnieke. Yeah. But you can follow your same protocol of work with kidney cells, and if you isolate a good batch, usually you get your offspring from it. Then you do the same thing again from another animal and you might not have it.

McWhir. Do you think this could be accountable by imagining that there are, perhaps, sub-karyotypic abnormalities in long-term tissue culture cells that aren't present in embryo-derived cells or primary cells?[51]

Schnieke. They're all primary cells.

McWhir. Then what do you mean when you say, 'when you find a cell line that works?'

Schnieke. We do a cell isolate and then we freeze them down, and when we have the cell isolate, it usually will give you offspring but the rate can still vary after your manipulation.

McWhir. So you haven't got a conventional cell line, but you do have a population of cells that have been multiplied in vitro?

Schnieke. Yes, because cell lines to me means that you have a cell which just grows and grows and grows, and of course those you can't use to make nuclear transfer animals.

Archibald. But your question still applies, Jim [McWhir], because those sub-karyotypic lesions could occur in the first couple of [developmental] passages, or they could be epigenetic.

Schnieke. Yes, but it is one of those questions where people have tried to get a handle on it, they have looked at the epigenetics, they have looked at methylation patterns, and it's just very difficult, because there's nothing really where you can afterwards say, if I do that test I can tell you that cell line goes or not. You can do rough things, like you can look at the chromosomal number and if all your chromosome sets are abnormal you don't have to carry on, but we don't even do that.

Lovell-Badge. In making iPS cells now people have tried to increase the efficiency, they use various things that can affect chromatin structures. Has anyone tried those sort of treatments?

Schnieke. We didn't, but some people have done something like this. Those experiments have been done. Did you do some [Bill Ritchie]?

Ritchie. Yeah, we looked at the visual appearance of cells and tried to correlate that with what came out at the end, and what we thought were nice cells proved to be really the opposite, and some of the cells which looked poor quality actually gave us some very good results as well. It just proved that we just don't know what is going on.

Colman. Ian, could I ask you what was Keith [Campbell]'s funding?

Wilmut. It was all one block of funding.

Colman. He wasn't a tenured person at Roslin, was he?

Wilmut. No.

Colman. I ask this because it always struck me that one of the lessons from that period was that in a university that could never have happened. No one would take on a project like that if it were going to be such a long project with large animals. You need institutes that have some sort of guaranteed funding over a relatively long term to actually encourage people to take the risk, because no normal post-doc would take that project on.

Archibald. Yeah, would be nice to move the [Roslin] Institute to that funding.

Sang. I think you would find that nobody would take on such a project now.

Colman. Is that the case?

Griffin. People come in on a three-year rolling programme. That is the standard institute model.

Colman. How do you get people in?

Griffin. That's been the great success of the Institute, in the past we've managed to do it. If you think of the breeding experiments on cattle, they weren't going to get any results for up to nine years, was it?

Sang. I wouldn't advise anybody to do the same [*laughs*] starting out now.

Griffin. But was that all on three-year contracts?

Sang. It was three to five.

Schnieke. It's the same for universities, we have the same problem. We get the same length of grant for people who do mouse work or just cell culture work, we get two to three years.

Colman. How do you persuade people who are just coming in, looking to build a career, that get a short-term grant with you. You can't guarantee they'll get another grant at the end, and you can't guarantee they're going to get any results in that first period – how do you actually get them to start?

Archibald. Well, you're asking that question in the context of Keith ... Keith jumped the fence and joined you guys [PPL]. And, arguably, with a start-up company you could look at a more precarious existence. But I think from what you said earlier it rings true that Keith went to Nottingham [in 1999, after leaving PPL] not because of the precariousness of the existence, but because he just liked the idea of being an academic scientist, and it was obviously a better place for that than anywhere else.

Sang. Keith had had a nonstandard career, because he did his PhD later, so he probably had fewer options, because it's very hard to make your career if you don't do a degree, a PhD, a couple of post-docs, in a nice linear fashion.

Ritchie. Just to take a step back, you were talking about the length of some of these experiments. When I started at the Stanhope farm, that was an experiment that ran for 25 years [*laughs*]: to alter or to look at genetics in large animals, you needed 25 generations. But as you say, Helen [Sang], we probably couldn't do the cloning experiments now, because a lot of the equipment that I used was actually made in our workshop. I haven't got a workshop [anymore], so it'd be really quite difficult doing it, it'd be almost impossible to actually carry out these sort of experiments, getting someone else to actually make the equipment that we first used, to actually make the pipettes and all the other paraphernalia that we

FIGURE 12. Four sheep cloned by Keith Campbell and his team at the University of Nottingham from the same cell line used to make Dolly. The sheep are Debbie, Denise, Dianna and Daisy. They were made in various studies between 2005 and 2007, and were announced in July 2016.
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required to do the cloning. It just wouldn't work at all.

Archibald. But that workshop effort was a joint enterprise between the Institute and the University, as far as I recall, and I'm not quite sure the university's got anything like that [today].

Sang. I think the Chemistry Department is about the only place that's still got a workshop.

Whitelaw. Just another general comment here, maybe Angelika can support it, Helen as well. It's not easy to recruit people, post-docs, into this field, and it's not only the duration [of the grants]: there isn't anywhere for them to go. We don't have open positions aplenty for aspiring group leaders to fill; it's still quite a small community. Having said that, as evidenced by this room, a lot of those groups are coming to the end of their careers, and maybe there'll be opportunities now [*laughter*].

Colman. You're talking about dead men's shoes now.

Whitelaw. But that's the real challenge, where do they go? I don't know how many groups we could classify as working with large animals, maybe ten, twelve at the most, around the world. Poultry, half of that, where do they go? They go into industry.

Lovell-Badge. Various things are starting to happen now, and going to the clinic, do you think that will expand the area, or is it always going to be small?

Whitelaw. Again, personally, I think most of that will go into the industrial environment.

Sang. I think that, although we went through a very lean period for agricultural research in the past, we came through. It still is very underfunded.

Lovell-Badge. In the UK?

Sang. In the UK and around the world. When you think about food security, the research input is relatively low given the challenges that we're facing.

Archibald. We've done the easy bits.

Schnieke. Yes, also the attitude of the general public towards GM food. Europe has kept the research on that area down. Now, it's sort of changing a bit, but up to now, it's the biomedical field where things have happened, and much less in agriculture.

Whitelaw. I want to back that, one of the reasons why it's quite good in the United Kingdom is that many of the big breeding companies are British, they may be international in their way, but they're British and this helps in our funding system.

McWhir. Just following on from your comment about the public perceptions of GM, it's interesting that, based on what I read, the basis of most public concern is aspects of genetically-modified crops that don't apply to farm animals. Now, there may be a completely different set of reasons for being frightened of genetically-modified farm animals, I would guess around animal welfare issues, but it's a completely different set of issues.

Schnieke. It is different between plants and animals, but on the animal side, like you said, it is more animal wellbeing and farming.

Colman. Unless it's fish like salmon.

Schnieke. Yeah, but how long did that [approval of transgenic salmon] take? With the farm animals, there is a public perception that we're mistreating the animals, there's no realistic picture of how agricultural animals are kept, and there is a sort of an idealistic vision of farm animals, and not a realistic one.

Griffin. If alpha-1-antitrypsin had worked, I don't think it would've had public resistance to it being introduced, so a key thing for the GM movement is to get one product that actually works.

Colman. I disagree.

Sang. I do, yes.

Colman. I think with the biomedical [issues], if you say there's an unmet clinical need and you can meet it, then people will think that's good.

Sang. Yes.

Colman. But with the food, they think, 'why do we need it'? They will need it, probably, when everyone's starving.

Sang. I give a lot of talks on the chickens and the applications. And there are medicines in eggs, really neat, resistance to bird flu, so people can conceptually think that these are all good applications, but actually when it comes to it, there's no real push to overcome the resistance to implementing these things. So it's a really complicated thing, and a lot of it in Europe is driven by the lobby groups, the NGOs who have the ear of the European parliament. I don't think it particularly reflects what 'the people' want, but they don't want the new things enough to overcome that. It all relates to conflating GM with multinational companies.

Lovell-Badge. But [GM animals] would solve quite a lot of the problems by having an organ transplant, right? It's bizarre.

García-Sancho. Something that strikes me when I read the annual reports of the Roslin Institute is that you can see the huge increase of publicity with Dolly, and you can track the media attention and how it becomes a lead story, whereas Polly receives very little space. If we look at this through the eyes of the pharming program, Polly was more like the conclusion of that program, because it was both genetically modified and cloned. I wonder why even in the internal publications of the Roslin Institute you see that difference in the amount of information.

Colman. I would say that Polly and Molly were incremental advances, despite the huge complexities in using nuclear transfer. Whereas the Dolly thing was a huge change.

Sang. But also, you're looking at the Institute reports. Those final steps weren't ours, they were within PPL. If we were reporting it now that we're attuned to reg-

istering the impact of our research, we might have more reach through to things that have gone from the Institute to application.

Colman. We didn't make any money.

Archibald. That's probably the crunch. These days we are expected to report impact of research and that's generally several years down the track.

Colman. When you say impact, what do you mean by impact?

Archibald. Socio-economic impact or impact on policy.

Colman. What about emotional impact?

Sang. That's not one of the headings.

Archibald. We continue to talk about the impact of the quantitative geneticist in terms of genetic improvement, we talk about the work that we've done in terms of detecting genetic variants that affect a certain vulnerability to infectious diseases, because we can quantify that they're making ongoing economic impact. At the time, we made a big noise about Dolly and PPL. These days we probably wouldn't repeat that because PPL unfortunately went bust, so that economic impact kind of stopped, as far as the bit that we could track. There are the bits that I'm not entirely sure we've got our hands on, in terms of the other things that happened post-Dolly, but we're probably not well placed to try and quantify that synthesis, what it delivered.

Colman. But if you were a pure research institute or an MRC institute, a major publication like that would be recorded very positively, long after. Even if it made no money, the impact it had on the way scientists worked elsewhere, a more altruistic, if you like, contribution

Sang. Well, we do that, too.

Schnieke. When it was rated Scientific Breakthrough of the Year, I think any institute would put it on their [publicity materials].

Sang. You can go down there to the University shop and buy a cuddly toy Dolly.

Lovell-Badge. Dolly the toy sheep, does Roslin get anything from that?

Archibald. The cuddly toy sheep, with its University sweater on [*laughter*], retails at … is it ten pounds?

Sang. Fifteen pounds!

Archibald. Fifteen pounds, and of that nine or ten are going into a fund that will be used to support post-docs and students going to conferences. It's the hottest selling item in the University of Edinburgh gift shop, apparently.

Colman. Will I be able to buy one in the airport?

Sang. No. The shop's at George Square if you've got time.

Lovell-Badge. So at least there is some economic benefit.

Griffin. We did consider whether or not we should trademark Dolly, a long while back, and trying to maintain a public image of the Institute that wasn't money grabbing, we made the decision not to.

Schnieke. Do you get a discount if you were involved? [*laughter*]

Archibald. I doubt it.

Sang. Go in the shop and see if you can try it!

Archibald. And there was of course the IKEA opportunity that you turned down, Harry.

Griffin. Which one was that?

Archibald. They wanted Dolly to open the IKEA store [*laughter*] and to dye her blue and yellow.

Griffin. We also turned down an invitation from Dolly Parton to ship the sheep to Dollywood.[52] Didn't think we would get it past the animal welfare committee.

García-Sancho. Maybe it is better to follow up on this over drinks! Before we finish, I just wanted to thank you very much for coming here, for making the effort, for all the contributions. It's been really enlightening for us.

Notes

1. Karl Illmensee announced the cloning of three mice at the University of Geneva in 1981, but was later accused of fraud and his results dismissed; see Gina Kolata, *Clone: The Road to Dolly and the Path Ahead* (London: Penguin, 1998), chapter 6. James McGrath and Davor Solter, who were key critics of Illmensee, were developmental biologists at the Wistar Institute in Philadelphia, and introduced key embryo manipulation techniques for nuclear transfer.
2. Neal First (1930–2014) was an American reproductive physiologist famous for his work with cattle, who cloned a calf from early embryonic (blastocyst) cells in 1994. Steen Willadsen is a developmental biologist who carried out various embryonic modifications in farm animals. He spent his early career at the Agricultural Research Council's Animal Research Station in Cambridge, but left for the United States after the station was merged with the Babraham Institute of Animal Physiology. Willadsen cloned a sheep from embryonic cells as early as 1984. By contrast with these experiments, Megan and Morag, born in 1995 at Roslin, were cloned from a cultured embryonic cell line, rather than cells taken directly from an embryo. The use of a cell line showed promise for precise genetic intervention.
3. John Clark (1951–2004) was a molecular biologist in charge of the genetic modification programme at Roslin, and director of the institute, 2002–04. Keith Campbell (1954–2012) was a cell biologist at Roslin and a key player in the sheep cloning experiments.
4. The 2007 Nobel Prize in Physiology or Medicine went to Mario Capecchi, Martin Evans, and Oliver Smithies for work on embryonic stem cells and their genetic modification that enabled gene targeting in mice, i.e. introducing precise genetic changes in specific parts of the genome.
5. Roger Land (1940–1988) was the director of ABRO and the Edinburgh Research Station of the IAPGR, 1982–88 (see Introduction, p. iv). He led the institutions through the dramatic cuts of the 1980s, and was responsible for establishing the pharming programme.
6. The Department of Trade and Industry set up a so-called LINK programme to fund farm animal embryo multiplication and provided half of the funds. The other funds came from the industry, specifically Animal Biotechnology Cambridge (a company established after Animal Research Station was merged with the Babraham Institute), the Milk Marketing Board, and Meat and Livestock Commission.
7. This part of the conversation, up to 'Colman. It's hard to know if...' (p. 3), was moved here from a later part of the discussion, in order to aid clarity and set up more context. This is once of two such transpositions in this transcript – see also note 16.
8. Dame Anne McLaren (1927–2007) was a prominent British developmental biologist. Her work, mostly done in mice, spanned many decades and focused on, among other topics, embryo transfer, chimaeric embryos, germ cells, and molecular aspects of mammalian development.
9. Sir John Gurdon is a British developmental biologist who cloned a *Xenopus laevis* frog from a somatic (i.e. non-reproductive) tadpole cell in 1958. He shared the 2012 Nobel Prize for Physiology or Medicine with Shinya Yamanaka 'for the discovery that mature cells can be reprogrammed to become pluripotent.'
10. Ralph Brinster is a US developmental biologist and reproductive physiologist at the University of Pennsylvania, who, in collaboration with Richard Palmiter at the University of Washington in Seattle, was among the teams that developed the first transgenic mice. Brinster's group went on to work on rabbit and farm animal transgenesis with scientists at the US Department of Agriculture's Agricultural Research Service laboratory in Beltsville, MA. See Robert E. Hammer et al., 'Production of Transgenic Rabbits, Sheep and Pigs by Microinjection,' *Nature* 315, no. 6021 (1985): 680–83. The 'News and Views' article mentioned is Robin H. Lovell-Badge, 'Transgenic Animals: New Advances in the Field,' *Nature* 315, no. 6021 (1985): 628–29.
11. Rick Lathe is a molecular biologist who was hired by ABRO in 1983 to set up their molecular biology programme. Before that, he had worked at Transgéne, a biotechnology company in Strasburg.
12. John Bishop is a molecular geneticist who worked at the University of Edinburgh. He collaborated with the ABRO pharming team in the 1980s, and trained John Clark, who took over the molecular biology programme at ABRO after Lathe left in 1986.
13. Richard D. Palmiter et al., 'Dramatic Growth of Mice That Develop from Eggs Microinjected with Metallothionein-Growth Hormone Fusion Genes,' *Nature* 300, no. 5893 (1982): 611–15.
14. Richard D. Palmiter et al., 'Metallothionein-Human GH Fusion Genes Stimulate Growth of Mice,' *Science* 222, no. 4625 (1983): 809–14.
15. Gordon Wright et al., 'High Level Expression of Active Human Alpha-1-Antitrypsin in the Milk of Transgenic Sheep,' *Nature Biotechnology* 9, no. 9 (1991): 830–34.
16. This part of the conversation, up to 'Harry Griffin. It might be useful ...' (p. 6), was moved here from a later part of the discussion in order to aid clarity. This is the second out of two such transpositions in this transcript – see also note 7.
17. There were arson attacks on IAPGR's Roslin site and a University of Edinburgh research site near Penicuik on 25 March 1989, linked to animal rights activists. Four firemen were injured.
18. Barry O. Hughes et al., 'Behavioural Comparison of Transgenic and Control Sheep: Movement Order, Behaviour on Pasture and in Covered Pens,' *Animal Science* 63, no. 1 (1996): 91–101.
19. GTC Biotherapeutics is based in Framingham, Massachusetts. In 2009 the US Food and Drug Administration approved antithrombin, a protein that prevents blood clotting, produced by GTC in transgenic goats.
20. Herman the Bull, the first transgenic bovine, was born in the Netherlands in 1990. He was created at the Dutch facilities of California-based GenPharm. The European arm of GenPharm has since become Pharming, Inc.
21. For a discussion of pharming approaches in plants see Richard Milne, 'Pharmaceutical Prospects: Biopharming and the Geography of Technological Expectations,' *Social Studies of Science* 42, no. 2 (2012): 290–306.
22. John W. B. King (1927–2006) was Director of ABRO, 1974–82. When ABRO's funding was cut in 1982, he was replaced

by Roger Land, a physiological geneticist charged with introducing molecular tools to the Organisation.

23. This refers to the long-term ABRO project studying the genetics of the Hereford cow breed. The project, which went on for many years and required heavy use of resources and land, represented the kind of agricultural research ABRO had to abandon after the financial cuts of 1982.

24. Charles (Charlie) Smith (1932–1997) was a pig breeder and human geneticist who spent most of his career at ABRO. Joining ABRO in the late 1950s, Smith designed schemes to improve practical pig breeding. In 1968, he moved to the University of Edinburghg Department of Human Genetics, where he worked on inherited human disease until his return to ABRO in 1974.

25. The Megan and Morag paper is Keith H. S. Campbell et al., 'Sheep Cloned by Nuclear Transfer from a Cultured Cell Line,' *Nature* 380, no. 6569 (1996): 64–66. The Dolly paper is Ian Wilmut et al., 'Viable Offspring Derived from Fetal and Adult Mammalian Cells,' *Nature* 385, no. 6619 (1997): 810–13.

26. The mass shooting at the Dunblane Primary School near Stirling occurred on 13 March 1996, when a gunman killed sixteen children and a teacher before killing himself.

27. Sir Alec Jeffreys is a British geneticist and Professor at the University of Leicester who developed key techniques for DNA fingerprinting, widely used in forensic science and paternity testing. See Michael Lynch et al., *Truth Machine: The Contentious History of DNA Fingerprinting* (Chicago: University of Chicago Press, 2010).

28. Esther N. Singer, et al., 'DNA fingerprinting Dolly,' *Nature* 394, no. 6691 (1998): 329–330.

29. Keith Campbell's team in Nottingham cloned four sheep from the cell lines used to make Dolly. These sheep were born in 2007, but only announced and published on in July 2016, shortly after this Collective Memory Event and Dolly's 20th anniversary. See Figure 11 (p. 39) and Kevin D. Sinclair et al., 'Healthy Ageing of Cloned Sheep,' *Nature Communications* 7 (2016): 12359, accessed on 17 March 2017.

30. Jaagsiekte is a contagious sheep lung cancer caused by JSRV (jaagsiekte sheep retrovirus). Dolly was euthanised on 14 February 2003 due to this disease.

31. Steve Connor, 'Dolly exposed as fake clone,' *Independent* 30 May 1999, accessed on 17 March 2017.

32. David Melton is a molecular geneticist at the University of Edinburgh. He was among the pioneers of gene targeting – specific genetic modification such as the removal of a gene – in mice, using embryonic stem cells.

33. Alexis Carrel (1873–1944) was a French surgeon who pioneered suturing techniques and tissue culture.

34. The Ministry of Agriculture, Fisheries and Food (MAFF) financed a considerable amount of agricultural research and its extension to farmers in the UK. Following Lord Rothschild's 1971 customer-contractor reforms, it became the key contractor for applied research in agriculture. In 2001, it was merged with the Department of the Environment, Transport and the Regions to create the Department for Environment, Food and Rural Affairs (DEFRA).

35. The Biotechnology and Biological Sciences Research Council (BBSRC) was formed in 1994 after the Agricultural and Food Research Council (AFRC) was merged with the biological sciences arm of the Science and Engineering Research Council. Being a research council, it is an autonomous body that makes decisions about sponsoring scientific research using government budget allocations.

36. Sir Martin Evans is a British developmental biologist who in 1981, with Matthew Kaufmann, isolated and cultured mouse embryonic stem cells. He shared the 2007 Nobel Prize in Physiology or Medicine with Mario Capecchi and Oliver Smithies, for enabling the creation of knockout mice and gene targeting.

37. The Scottish Development Agency (SDA, now Scottish Enterprise) was established in 1975 as a public body tasked with nurturing enterprise in Scotland through investment.

38. On Brinster's work with farm animals, see note 10.

39. Geron Corporation, founded in 1990, is a US biotechnology company based in Menlo Park, California. In 1999, Geron purchased Roslin Biomed, a company spun out of the Roslin Institute around the patents derived from the Dolly experiments.

40. The House of Commons Committee of Public Accounts reviewed the Geron deal in 2003, which involved public hearings where Grahame Bulfield and Harry Griffin testified. See House of Commons Committee on Public Accounts, *Reaping the Rewards of Agricultural Research* (London: The Stationary Office, Ltd., 2003), accessed on 15 March 2017.

41. 3i Group is a London-based private equity and venture capital company. It was founded in 1945 as the Industrial and Commercial Finance Corporation by the Bank of England.

42. Simon Best is a biotechnology expert who acted as CEO of Roslin Biomed. Before obtaining his MBA in 1985 and moving into biotechnology, he was a musician and ran a record label.

43. The British Technology Group (BTG) was founded as a non-statutory body with the 1981 merger of the National Research Development Corporation and the National Enterprise Board, to license and commercialise the use of publicly funded developments.

44. The Silicon Glen is a nickname for the high tech electronics sector of Scotland based mainly in the Central Belt triangle between Dundee, Glasgow and Edinburgh.

45. Supermice were large transgenic mice born after growth hormone gene injection (see note 13).

46. The knockout of alpha-1,3-galactosyltransferase enzyme in pigs using cloning was reported in 2002 by the US arm of PPL, and independently by a group from the University of Missouri: respectively, Yifan Dai et al., 'Targeted Disruption of the Alpha-1,3-Galactosyltransferase Gene in Cloned Pigs,' *Nature Biotechnology* 20, no. 3 (2002): 251–55; and Liangxue Lai et al., 'Production of Alpha-1,3-Galactosyltransferase Knockout Pigs by Nuclear Transfer Cloning,' *Science* 295, no. 5557 (2002): 1089-92.

47. Megan Davey is a developmental biologist at the Roslin Institute.

48. These papers are cited in note 46.

49. The first sheep gene targeting experiment, which involved specific insertion, was published as Kenneth J. McCreath et al., 'Production of Gene-Targeted Sheep by Nuclear Transfer from Cultured Somatic Cells,' *Nature* 405, no. 6790 (2000): 1066–69. The first sheep knockout results ap-

pear in Chris Denning et al., 'Deletion of the Alpha(1,3) Galactosyl Transferase (*GGTA1*) Gene and the Prion Protein (*PrP*) Gene in Sheep,' *Nature Biotechnology* 19, no. 6 (2001): 559–62.

50. The UK Human Fertilisation and Embryology Act was passed in 1990, in response to developments in IVF and pre-implantation genetic diagnosis. The Human Fertilisation and Embryology Authority that the Act created regulates reproductive technologies and experiments on human embryos. In 2001, the Human Reproductive Cloning Act was introduced to explicitly prohibit reproductive cloning in the UK.
51. Both primary cells and cell lines refer to cultured cells grown in vitro. Cell lines are established cells that have been continually propagated in culture over a long period of time, and have acquired homogenous genetic characteristics; primary cells are directly isolated from tissue and cultured.
52. Dollywood is a theme park in Tennessee jointly owned by Dolly Parton and Herschend Family Entertainment.

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